

No	Biomarker	Commercial laboratory available?	Analytical costs	Included in national monitoring program or surveys?	Marine, limnic or both?	Assessment criteria established?	SOP
				FR: considered for inclusion as part of D8 assessment under MSFD (Two MSs applied results from MN in mussels in the 2012 MSFD initial assessment)		<i>Mytilus edulis</i> (blue mussel), for gill 2.5% and for blood 2.5% <i>Mytilus galloprovincialis</i> (Mediterranean mussel), for blood 3.9 % <i>Mytilus trossulus</i> (bay mussel), for blood 4.5% <i>Platichthys flesus</i> (flounder), blood 0.3% <i>Limanda limanda</i> (dab), blood 0.5% <i>Zoarces viviparus</i> (eelpout) blood 0.4% <i>Gadus morhua</i> (cod) blood 0.4 % <i>Mullus barbatus</i> (red mullet) blood 0.3%	
18	Amphipod embryo malformation	?	Low	SE: Included in regular national monitoring of marine environment (Two MSs applied results in the 2012 MSFD initial assessment)	All but depends on species (in SE: <i>Monoporeia affinis</i> - available in Baltic Sea, but also lakes below highest coastline)	Yes, BAC and EAC values established. But method not included in ICES integrated strategy	TIMES, paper 41 ⁵⁴
19	Stress proteins	?	Low	?	All	No	Western blot or ELISA (?)
20	AChE	?	Low	Fulton and Key, 2001; Dellali et al., 2001; Fernando et al., 2005; Monteiro et al., 2007. Tested in SE	All, present in most Animals	Yes, both BAC and EAC, differs between species.	ICES TIMES 22 ⁵⁵

⁵⁴ Sundelin B et al., 2008. Biological effects of contaminants: the use of embryo aberrations in amphipod crustaceans for measuring effects of environmental stressors. ICES Techniques in Marine Environmental Sciences No. 41. 27 pp.
[http://ices.dk/sites/pub/Publication%20Reports/Techniques%20in%20Marine%20Environmental%20Sciences%20\(TIMES\)/times41/TIMES41.pdf](http://ices.dk/sites/pub/Publication%20Reports/Techniques%20in%20Marine%20Environmental%20Sciences%20(TIMES)/times41/TIMES41.pdf)

⁵⁵ Bocquene G, Galgani F, 1998. Biological effects of contaminants: Cholinesterase inhibition by organophosphate and carbamate compounds. ICES Techniques in Marine Environmental Sciences No. 22 [[HYPERLINK "http://ices.dk/sites/pub/Publication%20Reports/Techniques%20in%20Marine%20Environmental%20Sciences%20\(TIMES\)/times22/TIMES22.pdf"](http://ices.dk/sites/pub/Publication%20Reports/Techniques%20in%20Marine%20Environmental%20Sciences%20(TIMES)/times22/TIMES22.pdf)]

No	Biomarker	Commercial laboratory available?	Analytical costs	Included in national monitoring program or surveys?	Marine, limnic or both?	Assessment criteria established?	SOP
				ES: Included in MSFD monitoring and assessment (in fish and mussels) FR: considered for inclusion in D8 assessment under MSFD SE: Included in regular national monitoring of marine environment (applied in mussels in 3 MSs in the MSFD initial assessment; from 2012)			
21	Comet	?	Low/moderate	Pavlica et al., 2001; Buschini et al., 2004; Boettcher et al., 2010; Scaloni et al., 2010; Klobucar et al., 2010; Parolini et al., 2013 ?	All, limnic fish more frequently so far	Yes, BAC and depends on species: <i>Mytilus edulis</i> (blue mussel): 10%, <i>Gadus morhua</i> (cod): 5% and <i>Limanda limanda</i> (dab): 5%.	ICES TIMES 58 ⁵⁶
22	Mussel histopathology	?	?	?	? (also limnic mussels?)	BAC and EAC, varies between type of effect	No formal SOP established but a common reference in this context is Peters, 1988 ⁵⁷
23	Stress on stress	?	Low	ES: Included in MSFD monitoring and assessment (no MSs used in 2012 MSFD initial assessment but one MS had established targets)	? (also limnic mussels?)	Yes, BAC is 10 days and EAC is 5 days for <i>Mytilus</i> (blue mussels).	No formal SOP established but the method is considered very simple. A common reference in this context is Veldhuizen-

⁵⁶ Bean and Akcha, 2016. Biological effects of contaminants: Assessing DNA damage in marine species through single-cell alkaline gel electrophoresis (comet) assay. ICES Techniques in Marine Environmental Sciences No. 58, 21 pp. [[HYPERLINK "http://ices.dk/sites/pub/Publication%20Reports/Techniques%20in%20Marine%20Environmental%20Sciences%20\(TIMES\)/times58/TIMES%2058.pdf"](http://ices.dk/sites/pub/Publication%20Reports/Techniques%20in%20Marine%20Environmental%20Sciences%20(TIMES)/times58/TIMES%2058.pdf)]

⁵⁷ Peters EC, 1988. "Recent investigations of the disseminated sarcomas of marine bivalve molluscs. Amer. Fish. Soc. Spec. Publ.18: 74-92.

No	Biomarker	Commercial laboratory available?	Analytical costs	Included in national monitoring program or surveys?	Marine, limnic or both?	Assessment criteria established?	SOP
							Tsoerkan et al., 1990 ⁵⁸
24	SfG	?	?	ES: Included in MSFD monitoring and assessment (one MS used results in 2012 MSFD initial assessment)	? (also limnic mussels?)	Yes, BAC and EAC for <i>Mytilus</i> is 25 and 15 J h ⁻¹ g ⁻¹ respectively	ICES TIMES 40 ⁵⁹
25	Benthic diatom malformation	Yes	low	SE: No national survey done yet but regional campaigns performed in SE (see Kahlert 2012)	Limnic (both streams and lakes) but ture marine	Assessment criteria in SE, but to be used as risk indication (>2% malformations: risk)	Sampling and storage is standardised (EN 13946:2014). Method to identify malformations is included in "Undersökningstyp Påväxt i sjöar och vattendrag – kiselalgsanalys" ⁶⁰ & Kahlert M, 2012. See also Lavoie et al., 2017.
26	Egg shell thinning of bird eggs	No, not known	Very high (but difficult to separate costs from national monitoring of population)	SE: Included in regular national monitoring of marine environment (One MS used results in the MSFD initial assessment from 2012)	Marine	Assessment criteria in SE (for MSFD use): 0.59 mm (based on eggs sampled in 1856-1935)	Helander et al 2002

⁵⁸ Veldhuizen-Tsoerkan MBDA et al., , 1991. A field study on stress indices in the sea mussel *Mytilus edulis*. Application of the "stress approach" in biomonitoring. Arch. Environ. Contam. Toxicol. 21: 497-504.

⁵⁹ Widdows and Staff, 2006. BIOLOGICAL EFFECTS OF CONTAMINANTS: MEASUREMENT OF SCOPE FOR GROWTH IN MUSSELS. ICES Techniques in Marine Environmental Sciences No. 40, 34pp. [[HYPERLINK "http://ices.dk/sites/pub/Publication%20Reports/Techniques%20in%20Marine%20Environmental%20Sciences%20\(TIMES\)/times40/TIMES40.pdf"](http://ices.dk/sites/pub/Publication%20Reports/Techniques%20in%20Marine%20Environmental%20Sciences%20(TIMES)/times40/TIMES40.pdf)]

⁶⁰ [[HYPERLINK "https://www.havochvatten.se/download/18.6d9c45e9158fa37fe9f8d1a2/1482318545797/undersokningstyp-pavaxt-i-vatten-kiselalgsanalys-version-3-2.pdf"](https://www.havochvatten.se/download/18.6d9c45e9158fa37fe9f8d1a2/1482318545797/undersokningstyp-pavaxt-i-vatten-kiselalgsanalys-version-3-2.pdf)]

No	Biomarker	Commercial laboratory available?	Analytical costs	Included in national monitoring program or surveys?	Marine, limnic or both?	Assessment criteria established?	SOP
			productive parameters)				
27	Sea eagle productivity	No, not known	Very high (but difficult to separate costs from national monitoring of population productive parameters)	SE: Included in regular national monitoring of marine environment (One MS used results from "bird breeding success" in the 2012 MSFD initial assessment)	Marine	Assessment criteria established in HELCOM. Productivity: The threshold value is 0.97 nestlings. Brood size: The threshold value is 1.64 nestlings. Breeding success: The threshold value is 0.59 (59%).	Naturvårdsverket, 2004 HELCOM 2012 ⁶¹ In Sweden based on the assessment of nests 15 km or less from coast line
28	Pregnancy rate in seal	No, not known	Very high (but difficult to separate costs from national monitoring of population productive parameters)	SE: Included in regular national monitoring of marine environment (No MSs used results in the D8 MSFD initial assessment from 2012 but one MS defined GES and environmental targets for "reproductive health of marine mammals")	Marine	Assessment criteria established in Sweden (HVMFS 2012:18) for MSFD use (for grey seal in the Baltic Sea): good environmental status when pregnancy rate is above 80%.	Naturvårdsverket 2004 HELCOM 2012 ⁶²
29	Genes involved in xenobiotic biotransformation and regulation	?	Low	?	All	?	Scientific literature
30	Genes involved in oxidative stress, apoptotic	?	Low	?	All	?	Scientific literature

⁶¹ Naturvårdsverket 2004. Handledning för miljöövervakning. Undersökningstyp: Havsörn, bestånd. Programområde Kust och hav. Version 1:0: 2004-05-26. 2 HELCOM 2012. Baltic Sea Environmental Proceedings No. 129B. The development of a set of core indicators: Interim report of the HELCOM CORESET project. Part B. Descriptions of the indicators. Helsinki Commission. See also [[HYPERLINK "http://www.helcom.fi/Core%20Indicators/White-tailed%20sea%20eagle%20productivity%20HELCOM%20core%20indicator%202018.pdf"](http://www.helcom.fi/Core%20Indicators/White-tailed%20sea%20eagle%20productivity%20HELCOM%20core%20indicator%202018.pdf)]

⁶² Naturvårdsverket 2004. Naturvårdsverket 2004b. Handledning för miljöövervakning; Undersökningstyp: Patologi hos gräsäl, vikaresäl och knubbsäl. Programområde Kust och hav. Version 1:0: 2004-01-23. HELCOM 2012. Baltic Sea Environmental Proceedings No. 129B. The development of a set of core indicators: Interim report of the HELCOM CORESET project. Part B. Descriptions of the indicators. Helsinki Commission.

No	Biomarker	Commercial laboratory available?	Analytical costs	Included in national monitoring program or surveys?	Marine, limnic or both?	Assessment criteria established?	SOP
	response, Dna repair						
31	Mentum deformation in chironomids	Yes (?)	?	SE: used occasionally in the assessment of contaminated sites (sediments)	Limnic	No?	
32	Lipid peroxidation	?	Low	?	All	No?	Scientific literature
33	Protein carbonylation	?	Low	Prevodnik et al., 2007; Almroth et al., 2008; Parolini et al., 2013; Toni et al., 2011; Cattaneo et al., 2012	All	No?	Scientific literature
34	P-glycoprotein efflux	?	Low	?	All	?	Scientific literature

ANNEX III. Trigger value procedures

Sensitivity and specificity analysis of effect-based trigger-values (EBT) regarding the screening of known chemical and *in vivo* mixture risks

Background

This part presents a specificity and sensitivity analysis of *in vitro* EBM for the detection of ER-agonists in combination with effect-based trigger-values. The activation of the estrogen receptor by ER-agonists is a relevant mode of action that is related to adverse effects on the population level. The three watch list compounds estrone (E1), 17 β -estradiol (E2) and 17 α -ethinylestradiol (EE2) activate the estrogen receptor in an additive way. The respective EBT used for the assessment of the results obtained by *in vitro* EBM has to be defined in a way to maximise sensitivity and specificity for known mixture risks based on chemical analysis for the watch list compounds.

In addition to a specificity and sensitivity analysis with respect to chemical analysis, results obtained by *in vitro* EBMs are benchmarked as well against a transgenic fish model (*D. rerio*, EASZY assay Brion et al. 2017 and 2018 in prep) to characterise their predictive power for effects on higher biological levels and their potential to serve as an 'early warning' signal for *in vivo* effects. Although the stimulation of the estrogen receptor in brain tissue that is detected by the transgenic fish model is not an adverse effect per se it clearly demonstrates that estrogen receptor agonists present in a sample are bioavailable, taken up by the organism and distributed within the organism and across the blood brain barrier resulting in concentrations that are high enough to trigger the activation of the estrogen receptor in brain above control levels, possibly causing further effects in the fish.

The sensitivity and specificity analysis is based on published data for 33 surface- and waste water samples analysed within the EU estrogen monitoring project (see Kase et al. 2018, Könnemann et al. 2018) using five different *in vitro*-EBMs (ER α -CALUX, MELN; p-YES, Hela 9903 and ER GeneBlazer) and three chemical analytical methods based on hr-LC/MS for the quantification of E1, E2 and EE2. Furthermore, all samples were tested as well in a transgenic fish model (*D. rerio*, EASZY assay Le Fol et al. 2017 and Brion 2018 in prep). In previous studies it was demonstrated that the expression of the green fluorescence protein (gfp) fused to the cyp19a1b-gene reflects the behaviour of the endogenous brain aromatase gene in zebra fish (*D. rerio*, EASZY tg cyp19a1b-GFP transgenic fish line) and thus its brain specific response to hormonal regulation. By this means, this transgenic fish line allows the detection of ER-agonists in environmental samples including the toxicokinetics of compounds present in the sample. The induction of the brain aromatase gene is not yet an adverse apical endpoint per se but it clearly indicates the impact of ER-agonists on a key molecular initiating event in the context of a whole organism.

Methodology:

Step 1: The data from chemical analysis was used to calculate a chemical analytical cumulative risk quotient for each sample as follows:

$$RQ_{chem} = \frac{c_{E1}}{EQS_{E1}} + \frac{c_{E2}}{EQS_{E2}} + \frac{c_{EE2}}{EQS_{EE2}}$$

with

RQ_{chem}	cumulative risk quotient based on chemical analysis
c_i	concentration of the analytes E1, E2 and EE2 determined by hr-MS
EQS_i	proposed environmental quality standards for E1, E2 and EE2 (3600, 400 and 35 pg/L, respectively)

The rationale to calculate a cumulative risk quotient is the known additive behaviour of these three ER-agonists. The calculated cumulative risk quotients for the 33 samples are published in Kase et al. 2018. A cumulative RQ above 1 indicates a population relevant risk for aquatic species based on data from chemical analysis. The assessments based on *in vitro* results with different EBT scenarios were benchmarked against these cumulative RQs as described in ‘step 3’ (see below).

Step 2: The data from EASZY *in vivo* was assessed as follows:

If the EASZY-assay was stimulated significantly above the negative control (DMSO) in response to an exposure to the sample, the sample was defined as active, i.e. the risk quotient (*in vivo*) was >1. The concentration – response curves were modelled according to a Hill equation using the Regtox 7.0.6 Microsoft Excel TM macro⁶³, and EC20 values were calculated. For active environmental samples, the estrogenic activity is expressed as an E2-equivalent concentration (EEQ) using the ratio EC20 of E2/EC20 active sample.

The limit of quantification (LOQ) that defines as well the threshold above which samples were assessed as positive was calculated as follows: LOQ = mean GFP expression in DMSO controls + 3 x S.D. This was done by taking into account all the individual responses from all the DMSO controls (mean of the mean). The value was then expressed in ng E2/l by extrapolation to a mean E2 standard curve (obtained from all the E2 standard curves generated). The LOQ in terms of an E2 equivalence concentration and under consideration of an enrichment factor of 10 was determined as 6.3 ng/L E2 equivalents.

$$RQ_{in\ vivo} = \frac{BEQ}{AL}$$

with

$RQ_{in\ vivo}$	Riskquotient derived by <i>in vivo</i> analysis
BEQ	Bioanalytical equivalent concentration resulting from sample measurements
AL	Activation Limit for EASZY (6.3 ng/L E2 equivalents)

Step 3: Risk calculations of the selected *in vitro* EBM based on EBT

The results of *in vitro* EBM are also expressed in terms of a biological equivalence concentration (BEQ). In case of the selected *in vitro* EBM the results are provided as E2-

⁶³ http://www.normalesup.org/~vindimian/fr_index.html

equivalence concentrations (EEQ) in ng/l. The EEQ value represents the combined effect of all ER-agonists present in the sample. The EEQ value is compared to the EBT value in analogy to the chemical risk assessment.

$$RQ_{EBM} = \frac{EEQ}{EBT}$$

with

RQ_{EBM}	risk quotient based on <i>in vitro</i> EBM
EEQ	E2-equivalence concentration determined with an <i>in vitro</i>
EBM	
EBT	effect based trigger value

In recent publications EBT values for the assessment of estrogenic potentials in water samples were proposed (Jarosova et al. 2014, Kunz et al. 2015, van der Oost et al. 2017, Escher et al. 2018). These proposed EBT-values do not differentiate between various *in vitro* EBM that can be used for the detection of ER-agonists, i.e. all assay results are assessed against the same EBT-value. The use of one EBT for different EBM detecting the same MoA might be problematic because of EBM-specific differences in relative potencies for bioactive compounds. Therefore, a given EBT might be suitable for the assessment of one *in vitro* EBM but would be over-protective or under-protective in combination with another *in vitro* EBM. If possible, EBM-specific EBTs should be derived and tested for their performance against proposed generic EBTs.

In case of the estrogen receptor activation, alternative approaches are available to derive EBT-values that are specific for different *in vitro* EBM. One method is presented by Escher et al. (2018). The definition of EBT values is specific for a single *in vitro* EBM taking into account its performance characteristics such as limit of detection for model compounds and relative potencies of model compounds in relation to the reference compound E2. The specific EBT-values were determined by a read-across of published data. EBT values for the following *in vitro* EBMs were given by Escher et al. (2018): ER GeneBLAzer, Hela 9903, MELN and ERα-CALUX.

The second method proposed to derive EBM-specific EBT for estrogen receptor activation is based on the mean value of the above cited generic EBTs, i.e 400 pg/l EEQ. This mean EBT is modified based on the sensitivity of the *in vitro* EBM, its variability and relative potencies of prominent reference compounds. The details of this approach termed 'sensitivity factor approach' (SFA) are described in Annex III.3.

The selected proposals for EBT-values to assess estrogenicity in water samples are summarised below.

Table III.1. Proposed EBT-values in ng/l E2-equivalence concentration for the assessment of estrogenic potentials. na: not available.

<i>In vitro</i> EBM	Low generic	Median generic	High generic	Read across (RA) ⁴ specific	sensitivity factor approach (SFA) ⁵ specific
ER Gene BLAzer	0.3 ¹	0.4 ²	0.5 ³	0.340	0.400
Hela 9903				1.01	0.266
p-YES				na	0.266
MELN				0.370	0.266
ER α -CALUX				0.100	0.400

Step 4: Assessment of sensitivity and specificity

The assessment of the results obtained by the *in vitro* EBM by comparison to the different suggested EBT-values is benchmarked against the calculated cumulative risk quotient based on the chemical analysis (step 1) and *in vivo* results (step 2) in terms of true positive (tp), false positive (fp), true negative (tn) and false negative (fn)⁶⁴ test results as shown in Table 4 and 5.

Table III.2: Definition of true negative (tn), true positive (tp), false positive (fp) and false negative (fn) results with data from chemical analysis as reference point.

	$RQ_{chem} < 1$	$RQ_{chem} \geq 1$
$EEQ < EBT \rightarrow RQ_{EBM} < 1$	true negative (t _n)	false negative (f _n)
$EEQ \geq EBT \rightarrow RQ_{EBM} \geq 1$	false positive (f _p)	true positive (t _p)

An example of this benchmarking is shown in figure AIII.1 for EEQ-values obtained by the *in vitro* EBM ER α -CALUX with a generic EBT of 0.4 ng/l EEQ (= 400 pg/l). The R_{chem} values are given in log-space to achieve a symmetric representation of the values.

⁶⁴ It has to be pointed out that the categories true/false positive and true/false negative are defined based on the chemical analysis restricted to the target compounds E1, E2 and EE2. This assessment does not necessarily reflect the real risk associated with a water sample since further ER-agonists may be present that are not detected by chemical analysis. Thus, the assessment 'false positive' results from the comparison with R_{chem} that is an estimate of the real risk associated with a sample. The 'false negative'-results might be caused either by specific antagonistic compounds in the sample or by unspecific interferences with the *in vitro* EBM. In the first case the *in vitro* EBM would reflect the true estrogenic potential of the sample by taking agonistic and antagonistic mixture effects into account and the actual risk would be overestimated by the chemical analysis. The latter case would represent a real false negative test result and an existing risk would have been not detected by the *in vitro* EBM. In this respect sufficient control experiments and the definition of validity criteria are important to demonstrate the functionality of the *in vitro* EBM for a given sample. If validity criteria are not met, the sample cannot be assessed by the *in vitro* EBM. This situation is comparable to the presence of compounds interfering with a chemical analysis, e.g. due to ion suppression in mass spectrometry.

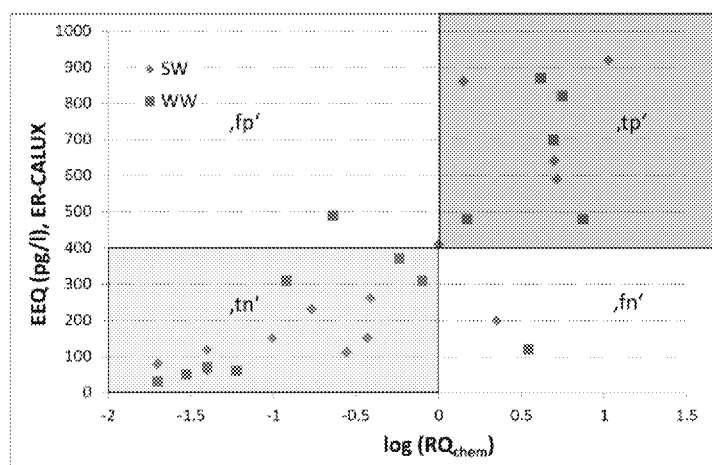


Figure AIII.1: Benchmarking of EEQ-values measured with the ER α -CALUX against RQ_{chem}. An EBT of 0.4 ng/l EEQ (= 400 pg/l) was used. True negative results are located in the green box, indicating no risk based on chemical analysis and the *in vitro* EBM. True positive results are located in the red box, indicating risk based on chemical analysis and the *in vitro* EBM. False positive results are located in the upper left part and false negative results are located in the lower right part of the diagram. SW:= surface water sample, WW:= waste water sample, tp: true positive, fp: false positive, fn: false negative, tn: true negative.

A higher EBT value would result in a lower number of 'false positive' results but in a higher number of 'false negative' results. The other way around: a lower EBT-value would result in a higher number of 'false positive' results but in a lower number of 'false negative' results.

The sensitivity and specificity for the various combinations of *in vitro* EBM with EBT are calculated as follows:

$$Y_{specificity}(\%) = \frac{t_n}{t_n + f_p} \cdot 100$$

$$Z_{sensitivity}(\%) = \frac{t_p}{t_p + f_n} \cdot 100$$

with

$Y_{specificity}(\%)$

$Z_{sensitivity}(\%)$

t_n

t_p

f_n

f_p

specificity in %

sensitivity in %

true negative, i.e. no risk indicated by chemical analysis and *in vitro* EBM

true positive, i.e. risk indicated by chemical analysis and *in vitro* EBM

false negative, i.e. risk indicated by chemical analysis but not by *in vitro* EBM

false positive, i.e. no risk indicated by chemical analysis but by *in vitro* EBM

The same approach as described above can be used to assess the sensitivity and specificity of the proposed EBTs in combination with the selected *in vitro* EMBs to predict effects in the transgenic *in vivo* model. The definition of negative (tn), true positive (tp), false positive (fp) and false negative (fn) is done in analogy to the benchmarking against chemical analysis as shown in Table 5.

Table III.3. Definition of true negative (tn), true positive (tp), false positive (fp) and false negative (fn) results with data from *in vivo* analysis with EASZY assay as reference point.

	$RQ_{in\ vivo} < 1$	$RQ_{in\ vivo} \geq 1$
$EEQ < EBT \rightarrow RQ_{EBM} < 1$	true negative (t_n)	false negative (f_n)
$EEQ \geq EBT \rightarrow RQ_{EBM} \geq 1$	false positive (f_p)	true positive (t_p)

Results:

The raw data for this analysis are available in a supplementary Excel file, this annex is focused on the presentation and discussion of the main findings of the sensitivity and specificity analysis.

The performance of the assessment based on *in vitro* EBM is based on the three parameters

- Sensitivity
- Specificity
- Variability of sensitivity and specificity between different *in vitro* EBM

using data from:

- chemical analysis and a
- transgenic fish model

as reference for benchmarking the predictive power of a given *in vitro* EBM / EBT-combination.

Sensitivity: A risk indicated either by the cumulated risk quotient using concentration data for E1, E2 and EE2 or by the activation of the transgenic fish model should be captured as well by the *in vitro* EBM. Otherwise the *in vitro* EBM would fail to detect samples that are defined as problematic by the reference approach.

Specificity: The *in vitro* EBM in combination with the EBT should only flag samples that were identified as problematic by the reference approach. Otherwise the *in vitro* EBM would overestimate the risk associated with a given sample.

Variability of sensitivity and specificity between different *in vitro* EBM: As described above some generic EBTs are proposed in different publications that are claimed to be applicable to all *in vitro* EBM for the same MoA. An EBT might fit well, i.e. high sensitivity and specificity, for a given *in vitro* EBM but is insufficient for another EBT. The variability reflects the applicability of an EBT to a range of *in vitro* EBM.

It is obvious that the parameters sensitivity and specificity have inverse tendencies. A very low EBT would result in 100% sensitivity, i.e. all samples assigned to be at risk by the reference approach were identified, but in 0% specificity because all samples assigned to be not at risk by the reference approach were identified as problematic by the *in vitro* EBM / EBT – combination. A very high EBT would result in an inverse situation with 0% sensitivity and 100% specificity. Because two categories have to be distinguished, the sensitivity and specificity of an *in vitro* EBM / EBT – combination has to be well above 50% to have any predictive power over flipping a coin. The optimal case would be a 100% sensitivity and 100% specificity. A balanced optimum would be an EBT that maximises sensitivity and specificity together.

A low variability of a generic EBT indicates a broad applicability of the proposed EBT for the *in vitro* EBM that were investigated. If the variability of proposed specific EBTs is lower, specific EBTs should be used to increase the predictive power of the *in vitro* EBM.

Figure AIII.2 summarises the results of the sensitivity and specificity analysis benchmarked against risk assessments based on chemical analysis (RQ(chem), top) and the use of the transgenic fish model (RQ(*in vivo*), bottom). The values for the proposed generic EBT-values are distinguished from those of the specific EBT proposals.

Table III.4. Sensitivities and specificities in % for five *in vitro* EBM detecting the presence of estrogen receptor agonists assessed by a proposed EBT-value of 0.4 ng/l E2-equivalence concentration (Kunz et al. 2015).

<i>in vitro</i> EBM	EBT [ng EEQ/l]	RQ(chem)		RQ(<i>in vivo</i>)	
		Sensitivity %	Specificity %	Sensitivity %	Specificity %
ER GeneBLazer	0.4	81.3	82.4	88.9	100
Hela 9903		75	94.1	72.2	100
p-YES		87.5	70.6	83.3	73.3
MELN		93.8	64.7	100	80
ERα-CALUX		87.5	94.1	83.3	100
Mean		85.0	81.2	85.5	90.1
%Cv		7.5	14.8	10.6	12.8

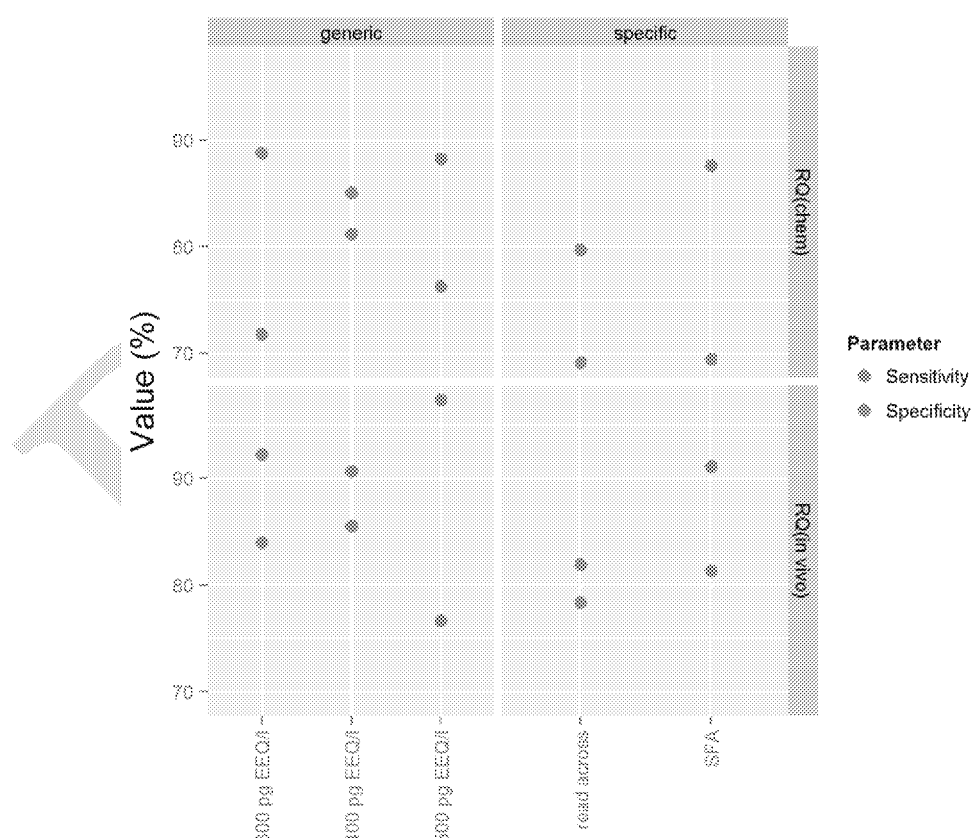


Figure AIII.2: Sensitivity and specificity analysis of *in vitro* EBM / EBT-combinations. Mean percentages for sensitivity (red dots) and specificity (blue dots) across all investigated *in vitro* EBMs are presented. Both parameters were calculated based on a comparison either with a risk assessment based on chemical analysis (RQ(chem), top) or results from a transgenic fish model (RQ(*in vivo*), bottom).

All EBT proposals proved to have a predictive power for the risk assessment based on chemical analysis of E1, E2 and EE2 as well as for the activation of the transgenic fish model (Figure AIII.2). However, specific differences in the performance can be observed. The mean sensitivity for RQ(chem) drops from about 89% in case of an EBT-proposal of 300 pg EEQ/l (Jarosova et al. 2014) to 76% in case of an EBT-proposal of 500 pg EEQ/l (van der Oost et al. 2017) whereas the mean specificity increases from 72% to 88%. Similar tendencies are to be observed in case of the benchmarking against RQ(*in vivo*). In this case the mean sensitivity drops from 92% to 77% whereas the mean specificity increases from 84% to 97%. The best balance between sensitivity and specificity is reached in case of an EBT-proposal of 400 pg EEQ/l (Kunz et al. 2015). The generic EBT proposal of 400 pg EEQ/l showed a higher concordance compared to the specific EBT-proposals. The mean sensitivity and specificity were higher in case of the generic EBT-proposal of 400 pg EEQ/l than for the EBM-specific EBT-proposals based on the read across approach. It has to be pointed out that in this case the calculated mean value was impacted strongly by one individual *in vitro* EBM, namely Hela 9903 with a proposed EBT of 1010 pg EEQ/l. In this case the sensitivity for RQ(chem) was only 38% and for RQ(*in vivo*) 33%. In contrast specificities were high with values of 100% each. This indicates that the proposed EBT for this specific *in vitro* EBM was probably too high. Compared to the sensitivity factor approach (SFA) described in Annex III.3 the generic EBT-proposal of 400 pg EEQ/l had a lower sensitivity but a higher specificity. This is most pronounced in case of RQ(chem) where the mean specificity for the SFA-approach is 70% and in case of the generic EBT-proposal 81%.

Figure AIII.3 shows the variability of sensitivity and specificity between different *in vitro* EBM assessed by the respective EBT-proposals.

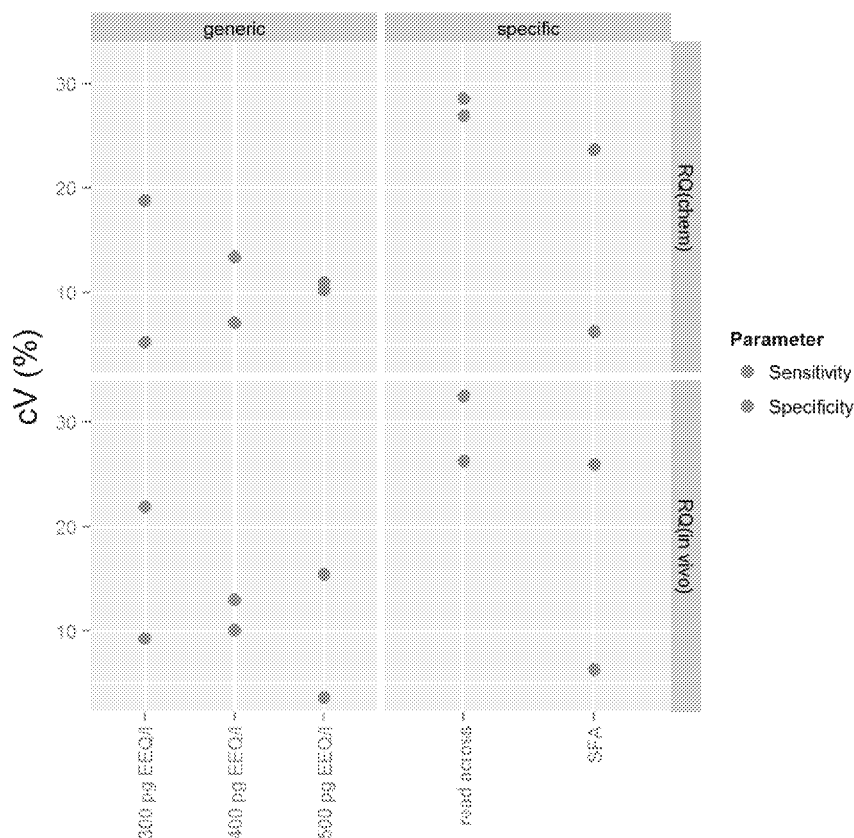


Figure AIII.3: Variability of sensitivity and specificity analysis of *in vitro* EBM / EBT-combinations. Variabilities for sensitivity (red dots) and specificity (blue dots) across all investigated *in vitro* EBMs are presented. Both parameters were calculated based on a comparison either with a risk assessment based on chemical analysis (RQ(chem), top) or results from a transgenic fish model (RQ(*in vivo*), bottom).

The lowest overall variabilities are observed in case of the EBT-proposals of 400 pg EEQ/l and 500 pg EEQ/l. In case of the sensitivity factor approach the variability for the determination of sensitivity was lower but in case of specificity higher. It has to be pointed out that the variability is not completely independent from the determination of sensitivity and specificity. In case of extreme EBT resulting in e.g. 100% sensitivity for all *in vitro* EBM the variability for the determination of the sensitivity will be 0%. Thus, the assessment of variability has to include both, sensitivity and specificity and has a meaningful outcome only in case of a EBT-proposal resulting in a balanced sensitivity and specificity. As discussed above generic EBT-proposals suffer from the inherent possibility that they might be not applicable to a selected *in vitro* EBM whereas performing well with another *in vitro* EBM. In the example presented here, the generic EBT-proposal of 400 pg EEQ/l performed best with respect to a balanced sensitivity and specificity performance and a low variability over a range of *in vitro* EBMs. Based on previous discussions with water experts this EBT was suggested in an international Estrogen monitoring recommendation as a moderate and balanced option as well (Dulio and Kase 2017). Nevertheless, it is important to have tools to derive specific EBTs as proposed by Escher et al. 2018 and the SFA described in Annex III.3 to derive EBM-specific EBT-values in cases where a generic EBT-proposal results in high variabilities.

Discussion:

As presented a sensitivity and specificity analysis can be done to assess the performance of proposed EBT-values in combination with *in vitro* EBM. This approach is able to elucidate the power of *in vitro* EBMs to assess in combination with EBTs the likelihood that a sample is at risk according to its chemical composition and/or the likelihood of the occurrence of an unwanted effect on a higher biological level. This type of analysis is easy to perform and is not based on any assumptions and independent from expert judgement. However, it requires the respective data obtained by *in vitro* EBMs, chemical analysis and/or *in vivo* EBMs. Such data sets are not yet available for most of *in vitro* EBMs but if an *in vitro* EBM is discussed as a possible candidate to be used as an element in water quality assessment it is recommended to perform a sensitivity and specificity analysis as outlined in this part.

In fact such data sets can be used as training sets to define optimal EBT-proposals. This is done by maximising sensitivity and specificity for the chemical risk, the possibility to observe effects on higher biological levels or both as illustrated in Figure AIII.4 and described in detail by Brion et al. 2018 (in preparation). As an example, EEQ values in pg EEQ/l obtained by the *in vitro* EBM 'ER CALUX' were used. The cumulated positive assessments by RQ(chem) and RQ(*in vivo*) were plotted against the log(EEQ). The first positive assessment based on RQ(chem) occurs at an EEQ of 120 pg EEQ/l, the second at an EEQ of 200 pg EEQ/l. Up to these EEQ-levels no positive *in vivo* result was observed. The highest EEQ at which no effect in the transgenic fish model was observed is 260 pg EEQ/l. The first positive result obtained by the transgenic fish model was observed at 310 pg EEQ/l. From 370 pg EEQ/l on the cumulated positive assessments for RQ(chem) and RQ(*in vivo*) increases. Thus, an EBT that differentiates best between positive and negative assessments by the reference methods lies between 260 and 310 pg EEQ/l. Based on this approach the EBT-proposal for the ER CALUX was set to an average value between these two EEQ-values.

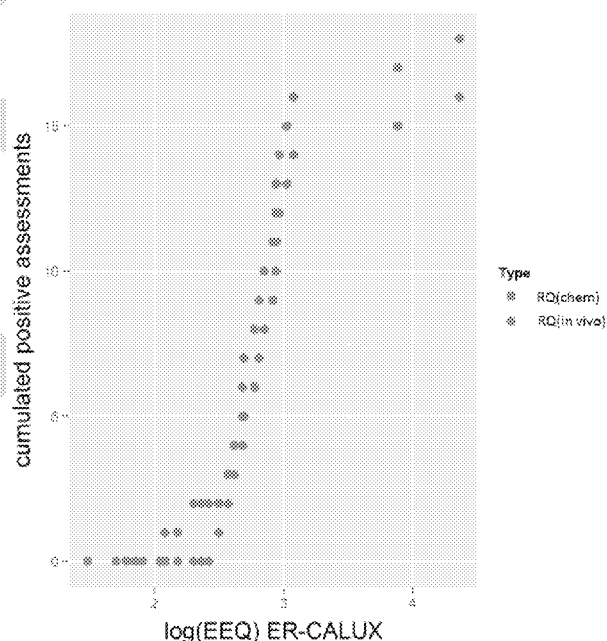


Figure AIII.4: Cumulated positive surface and waste water assessments vs. log (EEQ). The cumulated positive assessment of RQ(chem) (red dots) and RQ(*in vivo*) (blue dots) are plotted against the log(EEQ) measured by the ER CALUX.

The Table III.5 summarises the EBT-proposals for all investigated *in vitro* EBM based on this approach together with the respective values for sensitivity and specificity.

Table III.5: Proposed EBT-values in ng/l E2-equivalence concentration for the assessment of estrogenic potentials by Brion *et al.* 2018 and resulting sensitivity and specificity in %.

<i>in vitro</i> EBM	Brion et al. 2018	RQ(chem)		RQ(<i>in vivo</i>)	
		Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
ER GeneBLAzer	0.242	87.5	76.5	100	100
Hela 9903	0.182	93.8	82.4	93.3	94.4
p-YES	0.500	87.5	88.2	93.3	83.3
MELN	0.557	87.5	70.6	93.3	100
ER α -CALUX	0.283	87.5	76.5	100	100
Mean		88.8	78.8	95.5	96.0
%Cv		2.8	6.7	7.3	3.7

The EBT values shown in Table 6 result in the highest mean sensitivity and specificity. The variability between various *in vitro* EBM is comparable low. In sum the values indicate that it is possible to classify samples by means of *in vitro* EBM in good accordance to chemical analysis and results obtained by an organismic EBM. These proposed EBT-values showed the highest predictive power and are recommended for the assessment of the respective *in vitro* EBM for the detection of estrogen receptor agonists in water samples. However, these proposals have to be validated using an independent data set following the approach described above.

All EBT-values resulting from the different approaches lie within in a small range of EBT-proposals from 0.1 ng EEQ/l up to 1.01 ng EEQ/l. The majority of proposals are in the range from 0.18 ng EEQ/l up to 0.56 ng EEQ/l. The majority of proposed EBT-values were able to differentiate both, the exceedance of EQS-values for E1, E2 and EE2 and an effect induction on a higher biological level. This finding indicates a good overall consistency of the EBT-proposals.

Interestingly, the results from the *in vitro* EBMs show a higher agreement to the results obtained by the transgenic fish model in comparison to the results from the chemical analysis, i.e. the observed sensitivities and specificities for RQ (*in vivo*) are higher compared to RQ(chem) independent from the individual EBT-proposal. In fact four samples showed an RQ(chem) < 1 but were assessed as positive by the *in vitro* EBMs in most cases. According to the definition these assignments were 'false positive' results. A further sample showed a risk based on chemical analysis but in most cases this sample was identified as a negative result. However, the assessment of these samples by the *in vitro* EBMs was in good agreement to the classification based on the transgenic fish model.

This finding indicates that there is a possibility to underestimate a risk based on the chemical assessment. This reflects the need for a more holistic assessment of water quality because the chemical analysis of only three agonists of the estrogen receptor might not capture significant other agonists present in the environment. This leads inevitably to lower specificities if the results from the chemical analysis are defined as the 'true'

reference point. This example demonstrates the potential of *in vitro* EBM for a more holistic way to assess water quality as acknowledged by the EU Water Directors (WG Chemicals 2016).

Proposal of a tiered approach as a general framework to define EBT-values

The presented methods and concepts used for the definition of EBT-proposals and the evaluation of these proposals can be used to build up a framework for the definition of EBT-values based on available information to facilitate their use for e.g. prioritisation, screening or status assessment.

Similar to the definition of EQS as threshold values for chemical status assessments the derivation of EBT-values has to deal with inevitable uncertainties. As already discussed, uncertainties associated with the definition of EQS are caused by a lack of knowledge about the total composition of an environmental sample and possible mixture effects by the compounds present in the sample.

The used EBT derivation in this proposed concept is linked to EQS derivation which is protective for eco- and human toxicological risks according to the current knowledge level with the main difference that it also addresses unknown and unknown mixture risk and not only single substance-based risks.

EBM have the advantage that they cover mixture effects and effects of unknown contaminants in an environmental sample as they measure the integral effect that is caused by all compounds present in a sample. They can be used to address known and unknown mixture effects for population relevant effects (Kase et al. 2018). In case of biomarkers and many *in vitro* assays, specific molecular events are used as a marker for apical effects such as mortality, developmental or reproductive toxic effects. This can result in uncertainties about the translation from a molecular effect to an adverse outcome in the organism. Depending on the knowledge about the investigated mode of action (MoA) the level of uncertainty varies. For some MoA a link between *in vitro* results and adverse population relevant effects and risks can be established (Ankley et al. 2010, Matthiesen et al. 2017, Wittwehr et al. 2017, Kase et al. 2018).

A tiered approach for the derivation of EBT-values is proposed that is driven by the availability of data for the given MoA. This allows, on the one hand, the initial definition of EBT-values for a broad range of EBM to be used for prioritisation and screening purposes and, on the other hand, the subsequent refinement of EBT-values for prioritised EBM to reduce uncertainties of water body classifications. In general, uncertainties for both, EQS and EBT are reduced by an increased quality of the underlying data. The following flow chart outlines the suggested approaches for the derivation of EBT-values based on existing data.

EBT-values derived from the highest tier available are based on a broader data basis resulting in reduced uncertainties. Therefore, it is recommended to check data availability in advance and follow the flow chart from Tier 4 to Tier 1.

The decision for EBT derivation starts with testing the highest knowledge level Tier 4 downwards to Tier 1 as follows:

Tier 4: The most powerful data basis for the derivation of EBT-values is given by parallel *in vitro* and *in vivo* and chemical EQS compliance measurements. In other words, the *in vitro* effect quantified by an EBM is calibrated against mechanistically linked *in vivo* effects and quantified chemical mixture effects and risks (Brion et al. 2018 in prep.). A transgenic fish line is used for the detection of ER-agonists in environmental samples including the toxicokinetics of compounds present in the sample. This approach combines the established population relevance according to the chemical assessments of single compounds and direct *in vivo* results covering further unknown compounds with the same mode of action. By this means, the most direct link from *in vitro* results to unwanted endpoints of higher relevance and EQS compliance can be established. In principle, this approach can be transferred to other apical and adverse *in vivo* or *in vitro* effects of other MoA, e.g. PSII inhibition for herbicidal activity.

Advantages:

- Combines data from chemical monitoring and *in vivo* studies to define EBT-values with the highest discriminative power based on real environmental samples including mixture effects of known and unknown compounds.

Limitations:

- Comparatively high efforts and labour costs for the generation of the required data
- Transferrable to other MoAs if a suitable *in vivo* model is available
- Calibration was performed only against one *in vivo* method with its own strengths and weaknesses.

Conclusion: This approach links cell-based EBM to organismic EBM and data from chemical monitoring resulting in a robust EBT-value to differentiate between samples 'at risk' and 'not at risk'. Each EBM requires its own calibration with comparatively high efforts.

Tier 3: If both, data from chemical monitoring of compounds with an associated EQS-based mixture risk and results from an EBM are available for the same samples, the results from the EBM can be calibrated against the combined risk-quotient calculated for the detected compounds in the sample (Kase et al. 2018 and Könemann et al. 2018). Moreover, if EBM-specific knowledge of sensitivity, variability and relative potencies is available, the EBT can be adjusted to the uncertainty of used methods by the application of a sensitivity factor. This approach was discussed and prioritised by participants of an EBT workshop in Switzerland at the 22nd June 2017, in which some experts of the EBM task participated as well (<http://www.ecotoxcentre.ch/projects/aquatic-ecotoxicology/monitoring-of-steroidal-estrogens/>). For this approach, it is recommended to use the maximal sensitivity factor range of the respective EBM-specific EBT value according to Escher et al. 2018. The method is described in Annex III.4 in more detail.

Advantages:

- Based on EU EQS which indicate a population relevant risk level for many species establishing a relevant point of departure (POD)
- Only four EBM-specific parameters are necessary and can be transferred to other MoAs where information about EQS and EBM is available, e.g. photosynthesis II inhibition and dioxin-like effects.

- Simple to implement in regulation as the use of one screening EBT for each endpoint plus sensitivity factor will result in a low number of EBT which need to be implemented.

- Applicable with test specific knowledge, such as Limit of Quantification (LOQ), Coefficient of Variation (CV), and Relative Effect Potencies (REP) for all new and existing methods possible.

Limitations:

- Depends on the availability of high quality data, which is given only for selected, well characterised EBM
- Needs other approaches to set a first sensitivity range, but can be then applied independently.

Conclusion: This approach is recommended for all MoA for which no *in vivo* data are available, but for which EQS monitoring is successfully applied and sufficient knowledge about performance characteristics of the respective EBM is available.

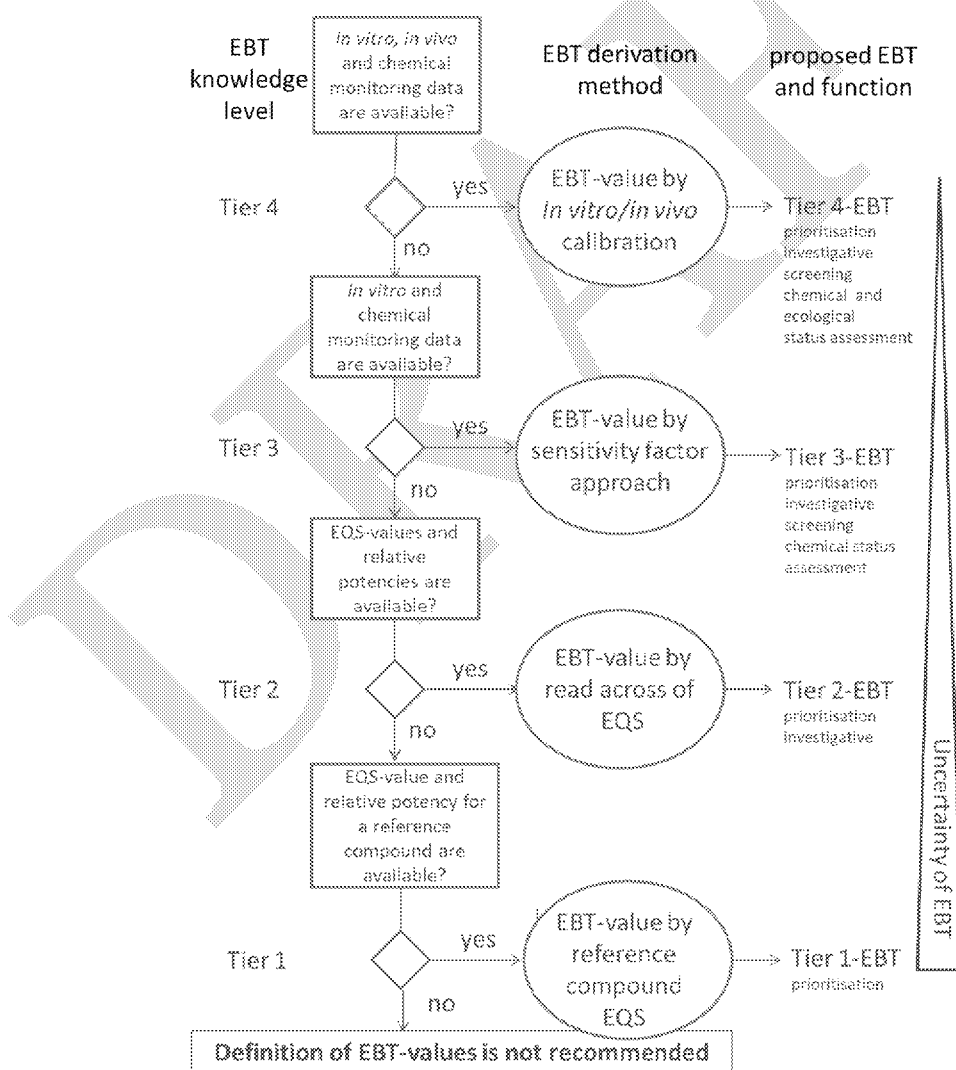


Figure AIII.5: Proposed concept of tiered Effect-Based Trigger value (EBT) derivation. EQS: Environmental Quality Standard.

Tier 2: If no experimental data from monitoring campaigns are available, EBT-values can be derived by a read across approach based on EQS-values of single compounds and the respective relative potencies of the compounds for the given EBM. (Escher et al. 2018). The proposed EQS-read across to define EBT-values was applied to a large number of EBM using more than individual 100 EQS-values (See Annex III.5).

Advantages:

- Based on multiple EQS indicating population relevant risk levels for many species establishing a relevant point of departure (POD)
- Can be applied for MoAs for which EQS and REPs of EBM are available
- Based on existing data resulting in an efficient and fast implementation.

Limitations:

- The approach depends on the quality and availability of data and possibly leads to higher uncertainties if only a limited number of compounds with associated EQS-values can be used for the EBT-derivation.
- Derived EBT depends on the selection of compounds to be included in the calculations. Stronger guidance is needed for the decision to select or de-select a compound for the EBT-derivation.
- Approach does not take into account EBM-specific inter-test CV and LOQs.

Conclusion: Recommended for all MoA for which no *in vivo* and chemical-analytical monitoring data are available.

Tier 1: If no read across approach is possible, the EQS of the reference compound in a certain mode of action (MoA), can be used as described above for an initial estimation of an EBT based on the respective EQS and the relative potency of the reference compound for the selected EBM. The reference compound should be either the most potent compound for the EBM or should be characterised as the main driver of the given biological effect in the environment. If no EQS of the reference compound is available, a certain BEQ level could be used instead of an EBT, but the interpretation of results may be weakened and is not recommended for EBT derivation.

Advantages:

- Very simple, the same concentration of the reference compound can elicit an adverse effect at EQS level
- Can be applied for many MoAs for which EQS are available.

Limitations:

- The method is not taking into account test-specific differences.
- The choice of the reference compound can largely influence results.

Conclusion: Only recommended for prioritisations of effect levels if no other EBT derivation method is applicable.

Safety and screening value of tiered EBT for surface water assessments – MoA ‘estrogen receptor activation’

The choice of EBT influences the safety and screening value of the EBM in surface water, illustrated as follows. The safety and screening value was calculated based on 80 surface water measurements performed in the estrogen monitoring project using 5 different EBM and compared to 48 high resolution LC/MS analytical measurements. For the calculations, the EBT-values derived for the four tiers (see annex 3) were used. The EBT-dependent risk indication for chemical analytical risks and the percentage of additional samples are summarised in Tables III.6 and III.7.

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Table III.6: Different Effect-Based Trigger value (EBT) approaches applied on results from measuring surface water (SW) samples with different effect-based methods (EBM) regarding risk indication and screening value (adapted from Kase et al. 2018)

EBM and condition	Tier 2: EBT Escher et al 2018 [ng/L]	Tier 2: Positive chemical analytical risk indication in SW	Tier 2: Additional positive samples without chemical analytical risk in SW	Tier 3: sensitivity factor approach [ng/L]	Tier 3 Positive chemical analytical risk indication in SW	Tier 3: Additional positive samples without chemical analytical risk in SW	Tier 4 : EBT Brion et al. 2018 [ng/L]	Tier 4: Positive chemical analytical risk indication in SW	Tier 4: Additional positive samples without chemical analytical risk in SW
ER GeneBlazer	0.340	7/7=100%	0/7=0%	0.400	5/7=71%	0/7=0%	0.242	7/7=100%	0/7=0%
Hela 9903	1.01	1/7=14%	0/7=0%	0.266	5/7=71%	0/7=0%	0.182	7/7=100%	0/7=0%
pYES	Na	na	na	0.266	6/7=86%	4/7=57%	0.500	6/7=86%	0/7=0%
MELN	0.370	6/7=86%	4/7=57%	0.266	6/7=86%	5/7=71%	0.557	6/7=86%	1/7=14%
ER Calux	0.100	7/7=100%	5/7=71%	0.400	6/7=86%	0/7=0%	0.283	6/7=86%	0/7=0%
Mean		75%	32%		80%	26%		91%	3%

Table III.7. Summary risk indication and screening properties of different Effect-Based Trigger value (EBT) approaches.

EBT option	Percentage of positive chemical risk indication of steroidal estrogens mixture risk for 16 surface water samples (cumulative RQ>1) in estrogen monitoring project	Percentage screening for other xenoestrogens: additional samples to analyse without known mixture risk of steroidal estrogens
Tier 1: Generic EBT = 400 pg/L*	77%	11%
Tier 2: EBT according to Escher et al. 2018**	75%	32%
Tier 3: sensitivity factor approach**	80%	26%
Tier 4: EASZY EBT approach**	91%	3%

*tested and published in Kase et al. 2018, ** calculated in annex 2

EBM data are validated against the risk identification based on high resolution LC/MS chemical analysis (risk identification and the low additional screening percentage for other xenoestrogens). The EBT derived from tier 4 resulted in the highest percentage of positive risk assessments and the lowest percentage of false positive risk assessments. The average percentage of positive surface water assessments decreases with decreasing tier that was used for the EBT derivation and the average percentage of false positive assessments increased with decreasing tier. In terms of safety the Tier 4 EBT are most appropriate, followed by tier 3 EBT. This result supports the tiered uncertainty approach in Fig. 1.

The situation at the moment is that the assessment using chemical monitoring data is accepted and implemented. By this means the respective data is a kind of an anchor for 'alternative' methods – such as EBM. However, with the current situation it is likely that an assessment based on an EBM will be compared to an assessment based on a chemical measurement. Especially, with respect to the possible application of EBM for screening (comparable to the use of EBM for "dioxins in food") it is necessary to "validate" the EBM-readout against the assessment based on chemical analysis (as assumed to be true). There would be no added value for the EBM (with respect to screening) if there is a high number of false negative and/or false positive assessments ("true" or "false" defined based on the outcome of the accepted chemical assessment and not necessarily "true" or "false" as an "absolute" assessment).

An application of the read across Tier 2 EBT shows that the very sensitive EBM have low EBT and the EBM with low sensitivity have high EBT. This leads – e.g. in case of the ER-CALUX – to a situation in which a high percentage of samples would pose an unacceptable risk although no risk is indicated by chemical analysis. This might reduce the acceptability of EBM. This is partially compensated with the Tier 3 proposed in the document that takes

into account low variability, sensitivity and relative potency and the proof of concept that the EBM have shown population relevant effects with high specificity and sensitivity.

Further data showing preliminary results from the ongoing effect-based watch list project are presented.

Preliminary results from the ongoing effect-based watch list project

In the course of the ongoing effect-based watch list project (presented at the last EBM meeting at the 2nd and 3rd October in Rome) further data was generated that support the findings described above. The following figure shows the BEQ for around 40 representative watch list water bodies investigated by the ER α -CALUX assay following ISO 19040-3.

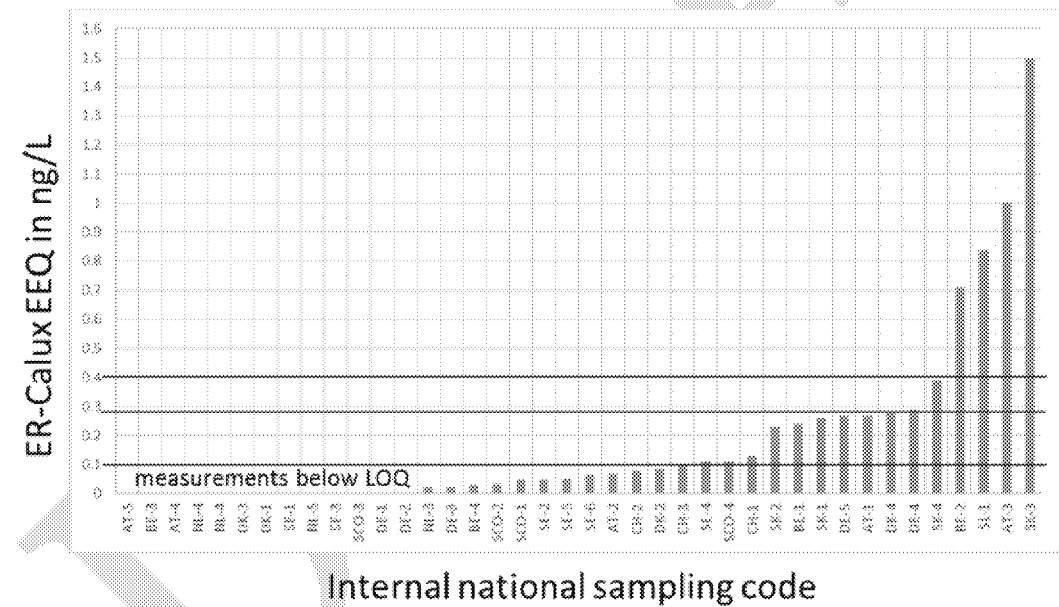


Figure AIII.6: Preliminary effect-based measurement data of around 40 EU watch list samples, measured with ER α -CALUX (LOQ were between 15 to 48 pg/L EEQ), investigated EBT are included as red lines.

Table III.8: Effect-based trigger value (EBT) exceedances for 40 watch list samples assessed using the EBT derived from the tiered approach.

	Tier 1	Tier 2	Tier 3	Tier 4
ER α -CALUX EBT	400 pg/L	100 pg/L	400 pg/L	283 pg/L
Percentage of positive watch list samples	10%	38%	10%	15%

Similar to the results of the estrogen monitoring project it can be also shown on representative watch list samples that an application of tier 2 EBT according to Escher et al. 2018 is leading to the highest percentage of positive samples with 38%, which would mean additional chemical analysis.

An application of tier 3 or tier 4 EBT would lead to 10-15 % of positive samples. In conclusion, the chemical analytical monitoring burden could be lowered remarkably by using higher tier EBT on representative samples. Higher tier EBT also have a good screening value in combination with standardised methods, e.g. ER α -CALUX method. Therefore, Tier 3 and 4 EBT are recommended additionally for screening.

Moreover, in a compliance check of 5 *in vitro* methods with 3 chemical analytical methods, it was shown on 33 water samples that an effect-based status assessment would be very useful because all samples can be classified as compliant or non-compliant with high specificity and sensitivity (Kase et al. 2018).

Both findings indicate a good screening and status assessment potential of different EBM for the MoA of ER receptor mediated estrogenicity by using higher tier EBT.

Description of sensitivity factor EBT approach (Tier 3)

Effect-based trigger (EBT) values are needed to assess if a sample poses an acceptable or an in-acceptable risk to the aquatic environment. EBT can be derived for certain endpoints or test-specific. Without EBT any applied inclusion of effect-based methods (EBMs) (e.g. for screening, prioritisation or status assessments) will be difficult to achieve in frame of the review of the EU Water Framework Directive (WFD).

Test specific EBT have the advantage that the specificity and sensitivity can be increased compared to endpoint specific EBT, which have to cover a broad set of different EBM. On the other hand, it is regulatory not feasible and meaningful to provide a separate EBT for each EBM as they can never keep up with the fast development of EBM and are very difficult to implement due to a large variability in available methods and potential new method developments.

The most preferred solution out of three options to derive EBT-values was discussed at the 22nd June at an EBT workshop in Dübendorf (CH) and furthermore discussed at the 2nd and 3rd October 2017 at the EBM-plenary meeting in Rome (IT).

This section describes the combination of an endpoint-specific EBT with a test-specific classification, based on its respective sensitivity, variability and specificity, for the MoA 'ER receptor activation'. This approach intends to combine the advantage of an easy to implement EBT derivation with a test-specific adjustment regarding specificity and sensitivity.

The EBM-dependent parameters are: the LOQ for the reference substance E2, the inter-test coefficient of variation (CV%) and the relative effect potencies (REP) for a less potent reference substance such as E1 and for a more potent reference substance such as EE2.

In a first step, data for 8 *in vitro* EBM (see table A1) were compiled, five of which were already characterised in the Estrogen Monitoring project (Kase et al. 2018). Three of the

selected EBM (A-YES, L-YES and ER-Calux) are standardised according to ISO (ISO 19040 parts 1 to 3).

Methods

Table III.9: Effect-based method (EBM)-specific characteristics for eight estrogen receptor (ER) activation assays*

E1 = estrone, E2 = 17 β -estradiol, EE2 = 17 α -ethinylestradiol, CV = coefficient of variation, REP = relative effect potency [LINK Excel.Sheet.12 "C:\\Users\\kaserobe\\Desktop\\June 2017\\Sensitivity factor chapter\\Sensitivity evaluation ER activation methods v4.xlsx" "Table 1!R1C1:R10C7" \a \f 5 \h * MERGEFORMAT]*corresponding data are in Kunz et al. 2017, Kase et al. 2017, CCVAM 2011, OECD 2009 or were provided by ISO contact points who are co-authors of this proposal. VM7Luc4E2 data were kindly provided by Timo Hamers from University of Amsterdam, NL.

**LOQs are calculated from sample concentrations. 3 x STDEV from the negative control with n=3 was the minimum LOQ requirement. The final LOQ was then divided by the relative enrichment factor (REF). REF = (SPE concentration factor (1000) / test specific dilution factor (x))

[LINK Excel.Sheet.12 "C:\\Users\\kaserobe\\Desktop\\June 2017\\Sensitivity factor chapter\\Sensitivity evaluation ER activation methods v4.xlsx" "Table 3!R1C1:R10C11" \a \f 5 \h * MERGEFORMAT]**Remark:** The VM7Luc4E2 is normally not working with 1000-fold enriched samples (as indicated in the table) and uses a maximal 250-fold enrichment, normally lower depending on the activity of samples. Moreover, VM7Luc4E2 has an additional enrichment step of 50 before 200-fold dilution. For other methods the REF might also be adapted regarding the activity of samples.

Starting from a generic screening-EBT value of 0.4 ng/L EEQ (see Kase et al. 2018), a maximum sensitivity factor of 4 can be estimated to address test-specific differences. This factor was used, as the maximal ratio between the lowest and highest EBT for the MoA 'ER-activation' published in Escher et al. 2018 and the generic screening-EBT of 0.4 ng/L BEQ is 4. The following classification scheme of sensitivity factors (see Table A2) was presented in June 2017 at an EBT workshop in Dübendorf (CH). This approach intends to simplify regulatory use and can be adapted with test specific EBT according to Escher et al. 2018 and with a test-specific sensitivity classification (see Table A3).

Table III.10: Proposal of a classification scheme of sensitivity factors for estrogen receptor (ER) activation.

LOQ = limit of quantification, E1 = estrone, E2 = 17 β -estradiol, EE2 = 17 α -ethinylestradiol, CV = coefficient of variation, REP = relative effect potency[LINK Excel.Sheet.12 "C:\\Users\\kaserobe\\Desktop\\June 2017\\Sensitivity factor chapter\\Sensitivity evaluation ER activation methods v4.xlsx" "Table 2!R2C2:R8C6" \a \f 5 \h * MERGEFORMAT]*If the rounded mean classification is exactly between 2 classes, e.g. between high (II) and moderate (III) it will be rounded to the lower mean (in this case moderate) sensitivity classification in order to increase the protectiveness. If only one parameter for one EBM is not available or out of range no sensitivity classification can be performed. This approach intends to stimulate minimum data availability and data quality for each EBM before using them for screening purposes and in combination with EBT. The sensitivity factor needs to be adapted for each relevant MoA according to available test specific EBTs calculated according to Escher et al. 2018

Results

The sensitivity categorisation scheme was applied for all 8 EBM to calculate a sensitivity factor. The results are shown in table A3. Four EBM (ER α -CALUX, A-YES, VM7Luc4E2 and ER-GeneBlazer) were ranked to the category 'high sensitivity' resulting in a sensitivity factor of 1. The other four EBM (Hela 9903, MELN, p-YES, L-YES Mc Donnell) were ranked to the category 'moderate sensitivity' resulting in a sensitivity factor of 1.5. The screening EBT value of 0.4 ng/L EEQ is modified by the test-specific sensitivity factor to allow a comparison of different EBT approaches as shown in table 2. Five of these EBM (No 1-5) were applied in the EU estrogen monitoring project and showed a good risk indication of steroidal estrogens compared to analytical results obtained by hr-LC/MS.

Table III.11: Sensitivity factor categorisation for the selected 8 effect-based methods (EBM) for the mode of action 'estrogen receptor (ER) activation'.

LOQ = limit of quantification, E1 = estrone, E2 = 17 β -estradiol, EE2 = 17 α -ethinylestradiol, CV = coefficient of variation, REP = relative effect potency, sensitivity classification: 1 (very high), 2 (high), 3 (moderate), 4 (low), 5 (very low) [LINK Excel.Sheet.12

"C:\\Users\\kaserobe\\Desktop\\June 2017\\Sensitivity factor chapter\\Sensitivity evaluation ER activation methods v4.xlsx"

"Table 3!R1C1:R10C12" \a \f 5 \h * MERGEFORMAT]

Four EBM (ER α -CALUX, A-YES, VM7Luc4E2 and ER-GeneBlazer) were ranked to the category 'high sensitivity' resulting in a sensitivity factor of 1. The other four EBM (Hela 9903, MELN, p-YES, L-YES Mc Donnell) were ranked to the category 'moderate sensitivity' resulting in a sensitivity factor of 1.5. The screening EBT value of 0.4 ng/L EEQ is modified by the test-specific sensitivity factor to allow a comparison of different EBT approaches as shown in table 2. Five of these EBM (No 1-5) were applied in the EU estrogen monitoring project and showed a good risk indication of steroidal estrogens with high-end chemical analytical risk.

Effect-Based Trigger value (EBT) compilation using the tiered EBT approach

Table III.12: EBT compilation using the tiered EBT approach, EBT in bold are proposed for use to the current knowledge level.

* UBA/JRC Dossier BPA 2016; ** Ecotox centre Dossier Clorpyrifos 2016; *** Ecotox Centre Dossier Diuron 2017; na: not available; dossiers available upon request. EBT values are not rounded and are shown as calculated and are not considering an accuracy of used methods or EBT. Data for Tier 2 are based on Escher et al. 2018.

N o	Measured endpoint or molecular target	Effect-Based Method /Assay name	Role in Adverse Outcome Pathway AOP	Reference compund	Tier 1 EBT [ng/L]	Tier 2 EBT [ng/L]	Tier 3 EBT [ng/L]	Tier 4 EBT [ng/L]	Comment
1	Activation of aryl hydrocarbon receptor (AhR)	H4L1.1c4 AhR assay	Toxicokinetics	Benzo[a]pyren e	50.000	6.358			
2	Activation of aryl hydrocarbon receptor (AhR)	PAH-CALUX	Toxicokinetics	Benzo[a]pyren e	50.000	6.205			
3	Activation of peroxisome proliferator- activated receptor (PPAR γ)	PPAR γ - GeneBLAzer	Toxicokinetics	Rosiglitazone	na	36.000			
4	Activation of peroxisome proliferator- activated receptor (PPAR γ)	PPAR γ -CALUX	Toxicokinetics	Rosiglitazone	na	data too preliminary to derive final effect threshold			
5	Activation of pregnane x receptor (PXR)	HG5LN-hPXR	Toxicokinetics	Di(2-ethylhexyl) -phthalate	1300.000	16273.280			
6	Activation of pregnane x receptor (PXR)	PXR-CALUX	Toxicokinetics	Di(2-ethylhexyl) -phthalate	1300.000	272494.999			

7	Activation of estrogen receptor (ER)	MELN	Hormone receptor regulation	17 β -Estradiol	0.400	0.368	0.266	0.557	
8	Activation of estrogen receptor (ER)	ER-GeneBLAzer	Hormone receptor regulation	17 β -Estradiol	0.400	0.337	0.400	0.242	
9	Activation of estrogen receptor (ER)	ERa_Luc_BG1	Hormone receptor regulation	17 β -Estradiol	0.400	0.625			
10	Activation of estrogen receptor (ER)	SSTA ER α -HeLa-9903	Hormone receptor regulation	17 β -Estradiol	0.400	1.008	0.266	0.182	
11	Activation of estrogen receptor (ER)	ER-CALUX	Hormone receptor regulation	17 β -Estradiol	0.400	0.104	0.400	0.283	
12	Activation of estrogen receptor (ER)	A-YES	Hormone receptor regulation	17 β -Estradiol	0.400	0.558	0.400		
13	Activation of estrogen receptor (ER)	3d YES	Hormone receptor regulation	17 β -Estradiol	0.400	0.882			
14	Activation of estrogen receptor (ER)	ISO-LYES (Sumpter)	Hormone receptor regulation	17 β -Estradiol	0.400	0.968			
15	Activation of estrogen receptor (ER)	VM7Luc4E2	Hormone receptor regulation	17 β -Estradiol	0.400	na	0.400		
16	Activation of estrogen receptor (ER)	p-YES	Hormone receptor regulation	17 β -Estradiol	0.400	na	0.266	0.500	
17	Activation of estrogen receptor (ER)	ISO-LYES ((McDonnell))	Hormone receptor regulation	17 β -Estradiol	0.400	1.068	0.266		
18	Estrogenic signalling	REACTIV (unspiked)	Hormone receptor regulation	17 β -Estradiol	0.400	0.797			
19	Antagonistic activity on the estrogen receptor (ER)	anti ER-GeneBLAzer	Hormone receptor regulation	Tamoxifen	na	currently not applicable because regulated chemicals are of low potency -> no read across possible			

20	Antagonistic activity on the estrogen receptor (ER)	anti ERa_Luc_BG1	Hormone receptor regulation	Tamoxifen	na	currently not applicable because regulated chemicals are of low potency -> no read across possible			
21	Antagonistic activity on the estrogen receptor (ER)	anti A-YES	Hormone receptor regulation	Tamoxifen	na	currently not applicable because regulated chemicals are of low potency -> no read across possible			
22	Activation of androgen receptor (AR)	AR-GeneBLazer	Hormone receptor regulation	Methyltrienolone (R1881)	na	currently not applicable because all regulated chemicals are of low potency (REP 1.10 ⁻³ to 1.2.10 ⁻⁵ compared to the hormone agonist R1881)-> no read across possible			

23	Activation of androgen receptor (AR)	MDA-kb2	Hormone receptor regulation	5 α -Dihydrotestosterone (DHT)	na	currently not applicable because all regulated chemicals are of low potency (REP 1.10 ⁻³ to 1.2.10 ⁻⁵ compared to the hormone agonist DHT)-> no read across possible			
24	Activation of androgen receptor (AR)	A-YAS	Hormone receptor regulation	5 α -Dihydrotestosterone (DHT)	na	currently not applicable because only two chemicals were active, which are also estrogenic at lower concentration			
25	Androgenic activity	RADAR (unspiked)	Hormone receptor regulation	17 α -methyl testosterone (17MT)	na	currently not applicable because none of the tested chemicals were active			

26	Antagonistic activity on the androgen receptor (AR)	anti AR- GeneBLazer	Hormone receptor regulation	Flutamide	na	3284.262			
27	Antagonistic activity on the androgen receptor (AR)	anti MDA-kb2	Hormone receptor regulation	Flutamide	na	3458.463			
28	Antagonistic activity on the androgen receptor (AR)	anti AR-CALUX	Hormone receptor regulation	Flutamide	na	14431.888			
29	Anti-androgenic activity	anti AR RADAR (spiked)	Hormone receptor regulation	Flutamide	na	3631.287			
30	antagonistic activity on the progestogenic receptor (PR)	anti PR-CALUX	Hormone receptor regulation	Endosulfan	5.000	1967.111			
31	Activation of glucocorticoid receptor (GR)	GR- GeneBLazer	Hormone receptor regulation	Dexamethasone	na	currently not applicable because all regulated chemicals are of low potency (REP 2.10^{-4} to 4.10^{-6} compared to the potent agonist dexamethasone) -> no read across possible			

32	Antagonistic activity of glucocorticoid receptor (GR)	anti GR-GeneBLazer	Hormone receptor regulation	Mifepristone	na	currently not applicable because all regulated chemicals are of low potency (REP 3.10^{-4} to 7.10^{-6} compared to the potent antagonist Mifepristone) -> no read across possible			
33	Competition with T4 for binding to transthyretin (TTR)	TTR RLBA	Hormone receptor regulation	Thyroxine (T4)	na	58.432			
34	Competition with T4 for binding to transthyretin (TTR)	TTR FITC-T4	Hormone receptor regulation	Thyroxine	na	49.153			
35	Modulation of thyroid hormone signaling	XETA (unspiked)	Hormone receptor regulation	Triiodothyronine (T3)	na	0.621			
36	Antagonistic activity on the thyroid receptor (TR)	Anti-TR-LUC-GH3	Hormone receptor regulation	Bisphenol A	240.000*	603.416			
37	Induction of oxidative stress response	AREc32	Adaptive Stress responses	Dichlorvos	0.600	155834.865			
38	Induction of oxidative stress response	AREGeneBLazer	Adaptive Stress responses	Dichlorvos	0.600	392090.410			
39	Induction of oxidative stress response	Nrf2-CALUX	Adaptive Stress responses	Dichlorvos	0.600	25579.901			

40	Growth inhibition	72h Algal growth inhibition	Population and organism response	Diuron	70.000** *	116.460			
41	Growth inhibition	24h Synchronous algae reproduction	Population and organism response	Diuron	70.000** *	109.362			
42	Growth inhibition	24h Combined algae assay (growth)	Population and organism response	Diuron	70.000** *	129.676			
43	Photosynthesis inhibition	2h Combined algae assay (PSII)	Population and organism response	Diuron	70.000** *	73.740			
44	Immobilization	48h Daphnia immobilization test	Population and organism response	Chlorpyrifos	0.460**	14.993			
45	Mortality after 48h	Fish embryo toxicity	Population and organism response	Bisphenol A	240.000*	275568.416			
46	Mortality after 96/120h	Fish embryo toxicity	Population and organism response	Bisphenol A	240.000*	182805.837			
47	Steroidogenesis modulation assay	H295 R	Steroidogenesis	Atrazine	600.000	na			
48	Steroidogenesis modulation assay	H295 R	Steroidogenesis	Forskolin	na	na			Forskolin is the most potent inducing compound

ANNEX IV. Integrated platform for EBM and application of Reference Materials

An integrated platform linking EBM to currently employed chemical and ecological assessment methods has been proposed in the JRC report on the integrated assessment of the current priority substances list under the Water Framework Directive and other substances of interest (Niegowska et al. 2018; Figure IV.1). Employed EBM selected based on endpoints most widely targeted by chemicals present in water bodies (e.g. oxidative stress, photosynthesis inhibition, endocrine disruption, carcinogenicity) would provide effect concentrations that, compared to reference materials, could be reported as EQS for a range of model organisms (Carvalho et al. 2014). Few endpoints would be sufficient to cover several mechanisms of toxic action with a precautionary principle so that mixture effects in biota can be timely prevented.

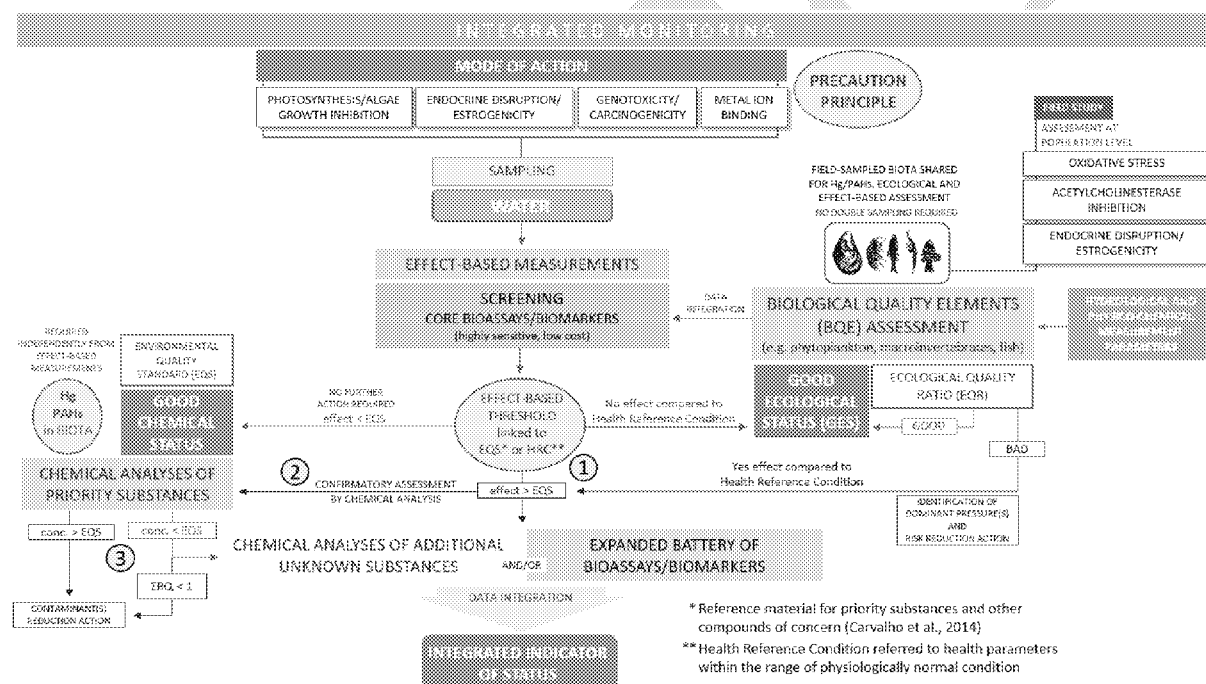


Figure IV.1: Framework for surveillance/operational monitoring linking EBM with chemical and ecological methods (Niegowska et al. 2018).

The purpose of this platform is to reduce the chemical assessment taking into account cost effectiveness without significantly impacting the entire workflow in terms of biological sampling which could be performed once for effect-based measurements, ecological assessment and detection of mercury (Hg) and polycyclic aromatic hydrocarbons (PAHs). Instrumental analysis is supposed to be executed for priority substances only in case of EBM results indicating effect concentrations above the safety threshold in order to confirm the presence of specific compounds and take contamination reduction action or perform additional analysis of unknown chemicals when measured substances do not exceed their EQS.

At population level, biomarkers relative to the most ecologically relevant endpoints (e.g. oxidative stress, acetylcholinesterase (AChE) inhibition, endocrine disruption) could be employed to inform about effects in biota compared to health reference conditions (HRC) corresponding to physiologically optimal parameters already defined to a large extent (e.g. normal AChE activity in flounder). Altogether, EBMs, ecological and chemical methods applied in a complementary manner according to the proposed platform would generate an integrated indicator of status as a holistic assessment of water quality and health conditions of biota exposed to realistically occurring chemical mixtures.

Example of a possible EU-wide exercise with Reference Materials

An approach evaluated recently in an EU-wide exercise proposed the use of a known chemical mixture with EQS available for each component as a reference material (RM) for EBMs (Carvalho et al. 2014). The RM compounds were selected based on their chemical structure and MoA to represent main pollutant groups found in surface waters which enabled the expression of results with reference to EQS even for unknown substances. Calibration curves generated from RM for a range of EBMs were used to extrapolate the obtained EC as EQS multipliers (xEQS) in a straightforward manner without the need to derive correction factors (Figure IV.2).

The availability of a standardised reference material is crucial to assess the performance of an EBM in a laboratory and should be used for a quality control along routine measurements and especially if a laboratory starts using an established EBM. If further EBM are developed in future, their performance could be benchmarked against this reference material.

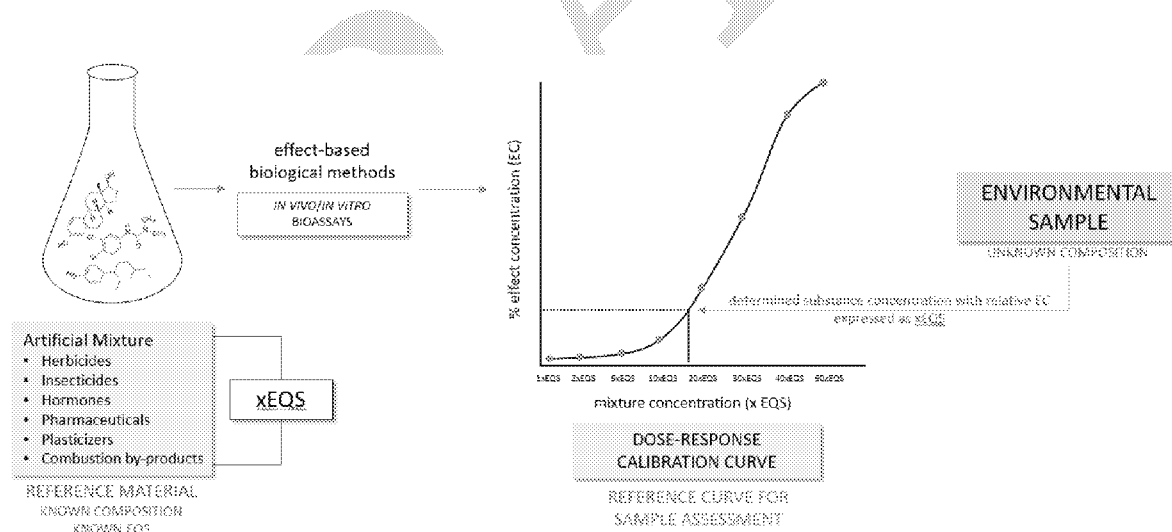


Figure IV.2: Workflow representing the approach based on the reference mixture proposed by Carvalho et al. 2014. Calibration curve generated from the RM at different concentrations is used to extrapolate the concentration of substances present in sampled water at which effects are induced.

The selection of chemicals for RM should be further investigated in order to identify differences in terms of assay performance based on the compounds included and to establish the most appropriate reference mixture composition for the effects to be assessed. Specific RM mixtures could be created for environmental sites where contamination by particular substances is expected or supposed, thus providing RM for surface water pollution profiling.

ANNEX V. Example of a Battery of EBM

The purpose of this annex is to (i) provide an overview of the recent bioanalytical test batteries typically used for water monitoring and assessment, and (ii) provide a recommendation for the use of a “standardised” bioassay battery for the evaluation of water quality.

Within the NORMAN Working Group (WG) 2 on Bioassays and Biomarkers in Water Quality Monitoring, in partnership with the SOLUTIONS project, a comprehensive review on the integration of bioassays and biomarkers in water quality monitoring and the selection of bioassays for a coherent battery of EBM was conducted [ADDIN EN.CITE ADDIN EN.CITE.DATA]. The bioassay batteries of different projects have been reviewed and compared in order to identify and to suggest a common battery of bioassays.

A recent NORMAN network interlaboratory study (ILS) verified whether a battery of miniaturised bioassays, conducted in 11 different laboratories following their own protocols, would produce comparable results when applied to evaluate blinded samples consisting of a pristine water extracts spiked with four emerging pollutants as single chemicals or mixtures [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Assays evaluated effects on aquatic organisms from three different trophic levels (algae, daphnids, zebrafish embryos) and mechanism-specific effects using *in vitro* estrogenicity (ER-Luc, YES) and mutagenicity (Ames, Ames Fluctuation) assays [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Within the SOLUTIONS project, Busch and co-workers [ADDIN EN.CITE <EndNote><Cite><Author>Busch</Author><Year>2016</Year><RecNum>14</RecNum>><DisplayText>[2]</DisplayText><record><rec-number>14</rec-number><foreign-keys><key app="EN" db-id="9s2xxp0auzoermeffsnvdvwjres5209swdes" timestamp="1529994910">14</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Busch, W.</author><author>Schmidt, R.</author><author>Schulze, M.</author><author>Altenburger, R.</author></authors></contributors><titles><title>Micropollutants in European rivers: A mode of action survey to support the development of effect-based tools for water monitoring</title><secondary-title>Environ. Toxicol. Chem.</secondary-title></titles><periodical><full-title>Environ. Toxicol. Chem.</full-title></periodical><volume>DOI:10.1002/etc.3460</volume><dates><year>2016</year></dates><urls></urls></record></Cite></EndNote>] systematically compiled organic contaminants detected in freshwater monitoring studies, provided an overview of the current knowledge available about the modes of action (MoA) for the detected compounds, performed a hazard ranking to identify priority mixtures, and reflected on the challenges in selecting appropriate bioassays for effect based monitoring. Furthermore, they suggested a list of organic compounds that could serve as a reference list for effect based methods validation studies [ADDIN EN.CITE <EndNote><Cite><Author>Busch</Author><Year>2016</Year><RecNum>14</RecNum>><DisplayText>[2]</DisplayText><record><rec-number>14</rec-number><foreign-keys><key app="EN" db-id="9s2xxp0auzoermeffsnvdvwjres5209swdes" timestamp="1529994910">14</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Busch,

W./author><author>Schmidt,
R./author><author>Schulze,
M./author><author>Altenburger,
R./author></authors></contributors><titles><title>Micropollutants in European rivers:
A mode of action survey to support the development of effect-based tools for water
monitoring</title><secondary-title>Environ. Toxicol. Chem.</secondary-
title></titles><periodical><full-title>Environ. Toxicol. Chem.</full-
title></periodical><volume>DOI:10.1002/etc.3460</volume><dates><year>2016</year>
</dates><urls></urls></record></Cite></EndNote>].

In the SOLUTIONS project a broad battery of *in vitro* bioassays based on human and fish cell
lines as well as whole organism assays using bacteria, algae, daphnids and fish embryos were
assembled for use in water quality monitoring [ADDIN EN.CITE
<EndNote><Cite><Author>Neale</Author><Year>2017</Year><RecNum>11</RecNum>
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timestamp="1529994910">11</key></foreign-keys><ref-type name="Journal
Article">17</ref-type><contributors><authors><author>Neale, P.
A./author><author>Altenburger, R./author><author>Ait-Aissa,
S./author><author>Brion, F./author><author>Busch,
W./author><author>Umbuzeiro, G. D./author><author>Denison, M.
S./author><author>Du Pasquier, D./author><author>Hilscherova,
K./author><author>Hollert, H./author><author>Morales, D.
A./author><author>Novak, J./author><author>Schlichting,
R./author><author>Seiler, T. B./author><author>Serra, H./author><author>Shao,
Y./author><author>Tindall, A. J./author><author>Tollefsen, K.
E./author><author>Williams, T. D./author><author>Escher, B.
I./author></authors></contributors><titles><title>Development of a bioanalytical test
battery for water quality monitoring: Fingerprinting identified micropollutants and their
Contribution to effects in surface water</title><secondary-title>Water
Research</secondary-title></titles><periodical><full-title>Water Research</full-
title></periodical><pages>734-
750</pages><volume>123</volume><dates><year>2017</year><pub-
dates><date>Oct</date></pub-dates></dates><isbn>0043-1354</isbn><accession-
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ISI>://WOS:000410010500071</url></related-urls></urls><electronic-resource-
num>10.1016/j.watres.2017.07.016</electronic-resource-
num></record></Cite></EndNote>]. The selection of bioassays was guided by the principles
of adverse outcome pathways in order to cover relevant steps in toxicity pathways known to
be triggered by environmental water samples. In a proof-of-concept study the effects of 34
water pollutants, which were selected based on hazard quotients, available environmental
quality standards and mode of action information, were fingerprinted in the bioassay test
battery. The proof-of-concept study not only demonstrated the utility of fingerprinting single
chemicals for an improved understanding of the biological effect of pollutants, but also
highlighted the need to apply bioassays for water quality monitoring in order to prevent
underestimation of the overall biological effect [ADDIN EN.CITE
<EndNote><Cite><Author>Neale</Author><Year>2017</Year><RecNum>11</RecNum>
<DisplayText>[4]</DisplayText><record><rec-number>11</rec-number><foreign-
keys><key app="EN" db-id="9s2xxp0auzoermeffsnvdvwjres5209swdes"
timestamp="1529994910">11</key></foreign-keys><ref-type name="Journal

Article">17</ref-type><contributors><authors><author>Neale, P.
A.</author><author>Altenburger, R.</author><author>Ait-Aissa,
S.</author><author>Brion, F.</author><author>Busch,
W.</author><author>Umbuzeiro, G. D.</author><author>Denison, M.
S.</author><author>Du Pasquier, D.</author><author>Hilscherova,
K.</author><author>Hollert, H.</author><author>Morales, D.
A.</author><author>Novak, J.</author><author>Schlichting,
R.</author><author>Seiler, T. B.</author><author>Serra, H.</author><author>Shao,
Y.</author><author>Tindall, A. J.</author><author>Tollefsen, K.
E.</author><author>Williams, T. D.</author><author>Escher, B.
I.</author></authors></contributors><titles><title>Development of a bioanalytical test
battery for water quality monitoring: Fingerprinting identified micropollutants and their
Contribution to effects in surface water</title><secondary-title>Water
Research</secondary-title></titles><periodical><full-title>Water Research</full-
title></periodical><pages>734-
750</pages><volume>123</volume><dates><year>2017</year><pub-
dates><date>Oct</date></pub-dates></dates><isbn>0043-1354</isbn><accession-
num>WOS:000410010500071</accession-num><urls><related-urls><url><Go to
ISI>://WOS:000410010500071</url></related-urls></urls><electronic-resource-
num>10.1016/j.watres.2017.07.016</electronic-resource-
num></record></Cite></EndNote>].

Based on the discussions within NORMAN and SOLUTIONS, a common battery of bioassays has been suggested that covers major toxicological endpoints. The recommended bioassay battery is also detailed in an upcoming Policy brief of the SOLUTIONS project (Figure V.1). It is suggested to complement *in vitro* assays by apical bioassays representing at least fish (fish embryo testing), invertebrates (*Daphnia*) and algae (cell multiplication inhibition) considered also as Biological Quality Elements (BQE) for pelagic communities in WFD. Of the MoA-specific *in vitro* assays, priority should be given to endocrine disruption and mutagenicity. Dioxin-like effects should be analysed particularly in sediments, biota and equilibrium passive samplers since typical drivers of these effects are very hydrophobic and accumulate in these matrices.

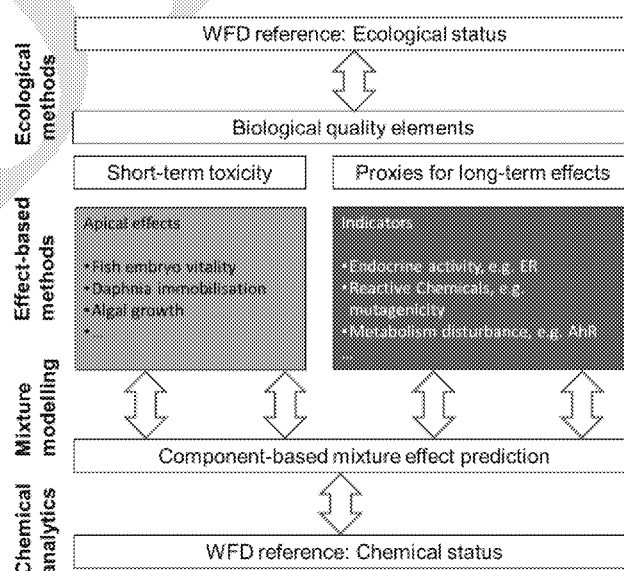


Figure V.1: Recommended test battery in the context of chemical and ecological status monitoring (redrawn from the SOLUTIONS Policy Brief Effect-based monitoring, Brack et al. 2018).

Literature:

[ADDIN EN.REFLIST]

ANNEX VI Neurotoxicity Outlook

Neurotoxicity was identified within the EU project SOLUTIONS as one of the most emerging modes of action in the environment. The numbers of potential neurotoxicants in the environment is raising and can pose a risk for humans and the environment. Considering the increasing numbers of environmental contaminants with potential neurotoxic potential, eco-neurotoxicity should be also considered in future risk assessments. In order to do so novel test systems are needed that can cope with species differences within ecosystems a selection of *in vitro* assays could be guided by Adverse Outcome Pathways (AOPs) relevant for eco-neurotoxicity. Currently the German Federal Ministry of Education and Research (BMBF) founded the project NeuroBox and the EU NORMAN network is performing a ringtest with neurotoxic substances considering behavioral changes in *Danio rerio*. Moreover, EURL ECVAM of the Joint Research Centre (JRC) is working on *in vitro* approaches to detect developmental neurotoxicity (DNT) triggered by a single chemical or in mixture.

For example, the JRC has developed human stem cell-based *in vitro* assays for evaluation of neurite outgrowth, synaptogenesis and neuronal electrical activity. This battery of assays in also included in ongoing EFSA/OECD DNT project which aims to develop a guidance document on use of DNT *in vitro* methods. The perturbation of these key neurodevelopmental processes (e.g. synaptogenesis, neuronal network formation and function) were identified as key events in several AOPs.

An evaluation of neurotoxicity (including developmental stage) is also be performed using non-mammalian species since the mechanisms underlying the development and function of the nervous system are well conserved across the phylogenic tree. Many of the basic molecular processes are identical in mammals and in non-mammalian species. Therefore, several alternative species including *Danio rerio*, *Oryzias latipes* or *Xenopus laevis* are used as vertebrate non-mammalian models and complementary to *in vitro* approaches. The small size, transparency during embryogenesis and speed of development make these species suitable for chemical testing. The gathering of data from these multiple information sources, could be used to develop Integrated Approaches to Testing and Assessment (IATA) designed in a fit-for-purpose manner for different regulatory purposes, including aquatic and human health protection. In the light of these ongoing developments a relevant selection of neurotoxicity assays for environmental assessments can be discussed at a later stage to advance the safety of assessments for neurotoxicity in the future.

Acknowledgement: Dr. Anna Bal-Price from EURL ECVAM at JRC for her support and knowledge sharing regarding ongoing neurotoxicity activities.

Message

From: Lynn L. Bergeson [lbergeson@lawbc.com]
Sent: 4/24/2018 3:23:37 PM
To: Morris, Jeff [Morris.Jeff@epa.gov]
CC: Henry, Tala [Henry.Tala@epa.gov]; Schweer, Greg [Schweer.Greg@epa.gov]; Fehrenbacher, Cathy [Fehrenbacher.Cathy@epa.gov]; Schmit, Ryan [schmit.ryan@epa.gov]; Vendinello, Lynn [Vendinello.Lynn@epa.gov]; Richard E. Engler, Ph.D. [rengler@lawbc.com]
Subject: New Chemical Issues--"Information flag"
Attachments: 00230086.pdf

Jeff,

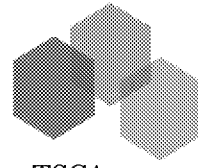
Thank you (and thanks to your colleagues) for meeting with Rich and me this morning. Our discussion was very helpful.

Supplementing our discussion about a possible "information flag," we attach the paper the New Chemicals Coalition (NCC) submitted on January 23, 2018, laying out a possible approach for communicating workplace exposure concerns identified as part of the new chemical review process. Rich and I will work on fine-tuning this paper to address more specifically the topics we discussed this morning. We believe, however, the concept outlined in the attached paper would go a long way in addressing the issues you noted.

Thanks again for meeting. We know how busy you and your colleagues are, and we appreciate the time you devoted to us.

Lynn

LYNN L. BERGESON
MANAGING PARTNER
BERGESON & CAMPBELL PC
2200 Pennsylvania Avenue, N.W. Suite 100W | Washington, D.C. 20037
T: 202-557-3801 | F: 202-557-3836 | M: 202-257-2872 | lawbc.com



TSCA
NEW CHEMICALS
COALITION

January 23, 2018

Via E-Mail

Jeffery Morris, Ph.D.
Director, Office of Pollution Prevention and Toxics
Office of Chemical Safety and Pollution Prevention
U.S. Environmental Protection Agency
1300 Pennsylvania Avenue, N.W.
Washington, D.C. 20004

Dear Jeff:

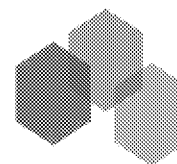
The Toxic Substances Control Act (TSCA) New Chemicals Coalition (NCC) submits this letter as a follow up to its December 1, 2017, letter related to the mandated consultation process with the U.S. Occupational Safety and Health Administration (OSHA) at TSCA Section 5(f)(5) (copy appended).

**Approach for Communication of Workplace Exposure Concerns
with New Chemical Notifier**

As discussed in the December 1, 2017, letter, and our other engagements with the U.S. Environmental Protection Agency (EPA), the TSCA NCC believes that EPA needs to implement an appropriately robust and ongoing consultation process with OSHA “prior to adopting any prohibition or other restriction” per TSCA Section 5(f)(5) that addresses occupational exposure issues. Should EPA identify new chemical risk concerns related to worker exposure issues in a premanufacture notice (PMN), EPA should evaluate the adequacy of the existing OSHA regulatory obligations and adopt additional restrictions or prohibitions only when needed to protect against unreasonable risks that are not otherwise addressed. Given the mandate to consult with OSHA, the proper role for EPA should be to provide hazard identification and risk assessment information to the PMN submitter, to OSHA, and to other potential manufacturers, importers, or processors to make these parties fully aware of EPA’s assessment and its identified occupational concerns, if any. Once informed of EPA’s assessment, the PMN submitter will be known to have information that must be considered in selecting respiratory protection and other personal protective equipment (PPE) needed to comply with OSHA’s broadly applicable regulations and with the General Duty clause requirement that

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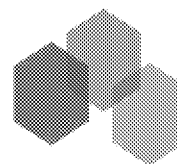
employers provide a safe working environment. By the same token, the TSCA NCC believes that once OSHA has been informed of EPA's assessment, it will be in a position to ensure that the General Duty clause requirements are being satisfied.

**Suggested Approach for Communication of Workplace Exposure Concerns
with Other Potential Manufacturers/Importers/Processors**

The TSCA NCC understands that in addition to communicating potential workplace exposure concerns to the PMN submitter, EPA must also have a mechanism to inform other potential manufacturers, importers, and/or processors of EPA's identified risk concern and the specific workplace exposure controls recommended by EPA to address that concern, including quantitative and qualitative exposure limits and suggested PPE.

The TSCA NCC proposed that this communication need can be addressed easily by using EPA's Inventory "flags." We know that before engaging in manufacturing, importing, or processing, a company must check the TSCA Inventory to confirm the subject chemical is listed on the Inventory and is active. EPA flags Inventory listings to indicate the existence of rules or other status. For example, the "T" flag indicates that the substance is subject to a test rule. An entity is thereby informed that a test rule has been promulgated and can research the terms of that rule. By flagging the subject chemical in the Inventory with a flag indicating workplace exposure concerns with a "W," EPA would appropriately inform the potential manufacturer, importer, or processor that a workplace exposure control issue has been identified and must be considered in the manufacturer, importer, or processor's OSHA compliance program. EPA can publish information concerning its occupational risk concerns and identified workplace exposure controls in a separate location, such as in the *Federal Register* or on EPA's ChemView database. Thus, the Inventory flag informs the potential manufacturer, importer, or processor that EPA has identified workplace risk concerns and the *Federal Register* or ChemView database can provide details on the concerns and the controls recommended by EPA.

As noted above and as EPA staff will recognize, this proposal for notification to potential manufacturers, importers, or processors builds off of existing EPA approaches. EPA already uses a number of Inventory flags to inform potential manufacturers, importers, or processors of critical information needed to comply with TSCA. For example, EPA uses Inventory flags to inform stakeholders of the existence of Section 5(e) or 5(f) orders, significant new use rules (SNUR), Section 6 rules, and Section 4 test rules. The flags apply to substances listed on both the public and confidential portion of the Inventory. As with the TSCA NCC



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proposal for workplace control flags, the current EPA flags do not indicate the specific details on the TSCA action, but require the manufacturer, importer, or processor to find those details in ChemView, the *Federal Register*, or the Code of Federal Regulations.

By building off of the existing Inventory flag approach, this proposal will be easy for TSCA stakeholders to understand and implement. Such an approach would also assist in identifying cases where OSHA may want to raise questions concerning compliance with its regulations and the general duty clause.

We hope you find this additional input helpful. We will be contacting your office soon to set up a meeting with you and your staff to discuss these ideas as well as our thoughts related to the use of polymer flags for listing of exempt polymers on the Inventory.

Sincerely,

Kathleen M. Roberts

Attachment

cc: Nancy B. Beck, Ph.D., DABT (w/attachment) (via e-mail)
Brian P. Grant, Esquire (w/attachment) (via e-mail)



December 1, 2017

Via E-Mail

Jeffery Morris, Ph.D.
Director, Office of Pollution Prevention and Toxics
Office of Chemical Safety and Pollution Prevention
U.S. Environmental Protection Agency
1300 Pennsylvania Avenue, N.W.
Washington, D.C. 20004

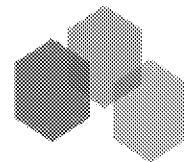
Dear Jeff:

This letter is submitted on behalf of the Toxic Substances Control Act (TSCA) New Chemicals Coalition (NCC), a group of representatives from over 20 companies that have come together to identify new chemical notification issues under the amended Toxic Substances Control Act (TSCA) and to work collaboratively with you and your team to address them. Thank you for the opportunity to meet on November 16; we appreciate the discussion that we had.

One of the topics that we raised concerned the mandated consultation process with the U.S. Occupational Safety and Health Administration (OSHA) at TSCA Section 5(f)(5), and the significance of restrictions included in the Safety Data Sheets (SDS) on new chemicals. As we discussed, the TSCA NCC believes that the U.S. Environmental Protection Agency (EPA) needs to implement an appropriately robust and ongoing consultation process with OSHA “prior to adopting any prohibition or other restriction” per TSCA Section 5(f)(5) that addresses occupational exposure issues. We believe that such a procedure is needed to ensure that EPA’s adoption of restrictions fully considers and avoids conflicts with OSHA’s established regulatory programs in addressing and mitigating worker exposure risks to new chemical substances, a result Congress seemed to intend in amending TSCA.

Picking up on a point raised in our meeting, we note for your information that EPA’s *Instruction Manual for Reporting under the TSCA § 5 New Chemicals Program*,¹ requires that the notification include, among others:

¹ Available at https://www.epa.gov/sites/production/files/2015-06/documents/instruction_manual_2015_5-26-2015.pdf.



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- A description of each specific worker activity during which workers may be exposed to the new chemical substance. Activities must be described even if workers wear protective equipment. The SDSs indicating recommended protective equipment should be submitted as part of Hazard Information in Part I, Section C, subsection 3 of the notice form.
- Information on the specific types of protective equipment and engineering controls that will be employed to protect the worker from potential exposure to the new chemical substance (*i.e.*, type of gloves, type of goggles, National Institute for Occupational Safety and Health (NIOSH)-certified 21c respirator, NIOSH-certified 19c respirator, closed containment system, nitrogen blanket, and related measures).
- Information on the physical form of the new chemical, the maximum number of workers exposed, and the maximum duration of exposure in hours/day and days/year.

The information elements noted above are not developed strictly for EPA review purposes. These information elements are required under OSHA which, as further articulated in the attached paper, has broad authority to regulate workplace exposures. Based on these reporting requirements for new chemical reviews, EPA staff will have access to available understanding concerning occupational exposures to the new chemical and the engineering controls or personal protective equipment (PPE) that the notifier believes is needed to protect workers, and on which the notifier will be regulated under OSHA.

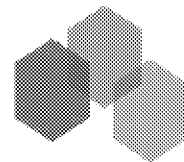
As discussed in more depth in the attached paper, the TSCA NCC does not believe that EPA's approach under TSCA adequately appreciates and recognizes the significance and effect of OSHA's statutory authorities and extensive regulatory scheme, as well as its enforcement mechanisms, governing workplace chemical exposures, including to new chemicals. These include:

- OSHA's detailed regulations for use of PPE when needed to further limit exposures beyond that afforded by OSHA's preferred approach of engineering and process controls. The regulatory standard, for example, requires use of respiratory protection to protect employees from exposure to air contaminants

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above an exposure limit, or where such protection is otherwise necessary to protect employee health. The standard places a range of OSHA enforced responsibilities on employers, requiring that a written program of respiratory protection must be in place including procedures for respirator selection, use, fit, testing, and so forth, training in use and hazards, and medical evaluations of employees who use such PPE.

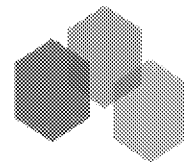
- The General Duty clause of the Occupational Safety and Health (OSH) Act that, among other provisions, requires every employer to furnish to each of its employees a workplace free from recognized hazards that cause, or are likely to cause, death or serious physical harm. The “likely to cause” aspect of the General Duty requirement is, as you recognize, particularly relevant to new chemicals given the limited information that is often available.

We believe that Congress did not intend to alter the scope of the effect of these OSHA requirements in amending TSCA. It, however, recognized the issue of overlapping authority concerning workplace regulation of new chemicals. For this reason, while additional authority was provided to EPA in making determinations and taking required actions, Congress included the OSHA consultation provision at Section 5(f)(5) to ensure that EPA’s regulation of new chemicals did not create or result in conflicts with requirements implemented by OSHA.

Although EPA has an obligation to review and make determinations regarding worker exposure issues and to formulate and adopt TSCA Section 5(e) actions that include measures to protect workers, this duty applies “to the extent necessary to protect against an unreasonable risk.” When this duty is juxtaposed with the mandatory consultation requirement, it is clear that EPA is required to evaluate the adequacy of the existing OSHA regulatory scheme and to adopt additional restrictions or prohibitions only when needed to protect against unreasonable risks not otherwise addressed.

Accordingly, the proper role for EPA should be to provide hazard identification and risk assessment information to the new chemical notifier and to OSHA to make these parties fully aware of EPA’s assessment and its identified occupational concerns, if any. Once informed of EPA’s assessment, the employer will be known to have information that must be considered in selecting respiratory protection and other PPE needed to comply with OSHA’s broadly applicable regulations and with the General Duty clause requirement that employers provide a safe working environment. By the same token, once OSHA has been informed of EPA’s

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assessment, it will be in a position to enforce its regulations and to ensure that the General Duty clause requirements are being satisfied.

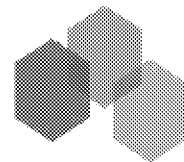
For these reasons, and others elaborated in the attachment, the TSCA NCC believes that EPA should disfavor issuing TSCA Section 5(e) orders that mandate use of particular PPE or other workplace-specific measures to mitigate occupational exposure. Instead, the TSCA NCC recommends the following approach if EPA identifies a workplace-specific risk concern:

1. EPA should consult with OSHA on the workplace risk concern.
2. EPA should inform the notifier of its assessment and concerns.
3. After the OSHA consultation and notifier communications are completed, EPA should no longer engage but instead rely on the employer's responsibilities mandated by OSHA, as well as OSHA's established expertise and robust existing regulatory program, to ensure worker protection.

Failure to follow a procedure as outlined above risks creating disputes over whether EPA's action preempted or created conflicts with OSHA's general authority and its regulations.

The TSCA NCC recognizes that the approach being advocated is at odds with EPA's longstanding practice in assessing and regulating new chemicals. Nonetheless, for the reasons provided above and elaborated in the attachment, TSCA NCC believes that EPA's prior and current approach is mistaken in that it does not give due recognition to OSHA's authorities and regulations and their role in ensuring a workplace free from recognized or potential occupational hazards. We believe that a modification in EPA's approach is necessary, given the changes in amended TSCA, including the OSHA consultation requirement. While EPA may have believed that, whenever an OSHA Permissible Exposure Limit (PEL) (or similar enforceable limit) is not in place, there is no enforceable requirement for companies to protect their workers from new chemical exposures, this belief is mistaken; and, as explained in this communication, does not have a basis in law or policy. Quite to the contrary, once EPA has informed the notifier and OSHA of its hazard and risk assessments, it has had the effect of triggering and setting in motion the existing regulatory requirements on employers to protect workers from recognized or likely occupational harms. Thus, any belief by EPA that, in the

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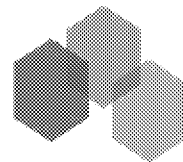
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absence of a TSCA Section 5(e) or Significant New Use Rule (SNUR) requirement to protect workers, it cannot ensure the presence of an enforceable regime of workplace protections is in fact a mistaken and erroneous belief.

Put another way, EPA's current practice under amended TSCA to equate any potential health hazard to represent an unreasonable and unmanaged risk to potentially exposed workers represents a misreading of the broadly applicable and pervasive regime that is implemented and enforced based on the OSH Act and OSHA's regulations and policies. On the contrary, once appropriately informed of EPA's concerns, any employer having a commercial relationship to the notifier must be made aware of and must consider EPA's assessment conclusions and respond appropriately to meet their obligation to protect workers and provide for a safe workplace. Furthermore, the fact that OSHA has also been informed of EPA's concerns puts to rest any questions about the level of information and the hazard, exposure, and risk assessments that the notifier and affiliated employers have access to, and establishes a factual written record that can be considered during any OSHA inspections or enforcement actions.

The TSCA NCC believes that for many, if not most, new chemicals for which EPA has proposed workplace restrictions under new TSCA, once EPA has informed OSHA and the notifier of its occupational risk assessment, that will be sufficient to ensure adequate workplace protection and to make any unreasonable risk to workers "not likely." Having made such a determination regarding occupational risks, EPA should proceed to meet its obligations to assess and determine other exposure risks, such as to the environment and general population, and to take the steps required depending on the final determination. Such a change in EPA's approach would avoid the issues associated with overlapping authority and imposing duplicative, if not conflicting, requirements for workplace exposures while also allowing EPA to focus its regulatory resources on other potential risks that are not subject to the overarching and comprehensive requirements that otherwise apply in the workplace.



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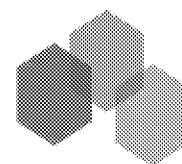
We hope you find these comments helpful. We would be pleased to discuss them with you and your staff in more detail prior to the **December 6, 2017**, public workshop if that is of interest.

Sincerely,

Kathleen M. Roberts

Attachment

cc: Nancy B. Beck, Ph.D., DABT (w/attachment) (via e-mail)
Kevin W. McLean, Esquire (w/attachment) (via e-mail)
Brian P. Grant, Esquire (w/attachment) (via e-mail)



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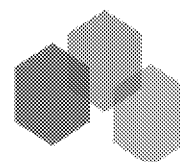
**TSCA New Chemicals Coalition¹ Position Statement Concerning the
Consultation with OSHA Required by New TSCA and EPA
Adoption of Restrictions to Address Workplace Exposures
December 2017**

I. ISSUES TO BE RESOLVED

The TSCA that was originally enacted in 1976 was comprehensively restructured and revised in 2016 by the Frank R. Lautenberg Chemical Safety for the 21st Century Act (new TSCA). New TSCA generally requires EPA to issue an order under Section 5(e) governing the manufacture, processing, distribution, use, or disposal of a new chemical substance whenever EPA makes a determination under Section 5(a)(3)(B). EPA is directed to “prohibit or limit the manufacture, processing, distribution in commerce, use, or disposal of such substance ... to the extent necessary to protect against an unreasonable risk of injury to health or the environment.” As part of this determination, EPA must consider risks “to a potentially exposed or susceptible subpopulation” that EPA deems relevant, which typically will include workers who are occupationally exposed to the new substance during the manufacture, processing, or use of the substance.

While EPA may issue an order under new TSCA Section 5(e) that contains prohibitions or restrictions intended to address workplace exposure, new TSCA Section 5(f)(5) requires that, prior to doing so, “[t]o the extent practicable, [EPA] shall consult with the Assistant Secretary of Labor for Occupational Safety and Health....” This required consultation with the U.S. Occupational Safety and Health Administration (OSHA) is vital as it both acknowledges a role for EPA concerning workplace exposures and explicitly recognizes OSHA’s primary responsibility for protecting worker safety and health. TSCA NCC believes that the clear intent of the consultation provision is to require that EPA, before deciding to implement separate TSCA action, will jointly evaluate the contemplated regulatory approach with OSHA, thereby assuring that EPA adequately considers OSHA’s established regulatory programs and avoids conflicts or confusion in addressing and mitigating worker exposure risks to a new chemical substance. Section 5(f)(5) addresses the need for consultations “prior to adopting *any* prohibition or other restriction” (emphasis added). Without such ongoing consultations, TSCA NCC believes that EPA’s adoption of restrictions for a new chemical to

¹ The Toxic Substances Control Act (TSCA) New Chemicals Coalition (NCC) is a group of representatives from over 20 companies that have come together to identify new chemical notification issues under the new TSCA and to work collaboratively with the U.S. Environmental Protection Agency (EPA) to address these issues.



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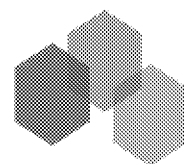
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address workplace exposures that are also regulated by OSHA would inevitably increase the potential for conflicts concerning -- or material differences in interpretation -- of these parallel requirements.

The value of coordination was recognized in a 1981 Memorandum of Understanding (MOU) between EPA's Office of Prevention, Pesticides, and Toxic Substances (OPPTS), a predecessor of the current EPA Office of Chemical Safety and Pollution Prevention (OSCPP) (attached). The MOU included provisions relating to sharing of information and joint participation in reviews and regulatory determinations on new chemicals presenting an occupational concern as well as sharing of confidential business information (CBI).

Section 4(b)(1) of the Occupational Safety and Health (OSH) Act, which addresses preemption of OSHA's regulatory authority in certain instances, states: "Nothing in this Act shall apply to working conditions of employees with respect to which other Federal agencies ... exercise statutory authority to prescribe or enforce standards or regulations affecting occupational safety or health." An MOU entered into by EPA and OSHA on February 13, 1991, affirms that OSHA retains principal "broad authority" to regulate workplace exposures to chemicals, while "EPA responsibilities include the protection of public health and the environment." The comprehensive OSHA Field Operations Manual (FOM) (2016), in explicitly addressing the effect of this preemption provision, observes that the only group of workers for whom OSHA regulation is considered to be preempted by EPA authority are farmworkers and pesticide applicators directly exposed to pesticides registered under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), for which the worker protection measures in the EPA-approved label instructions preempt OSHA. While changes in the regulatory landscape may change the scope of preemption as well, as the FOM notes, how it is delineated can be a complex determination. It would be inappropriate for EPA to presume that it has been afforded broad authority under TSCA Section 5(e) to make independent regulatory decisions affecting areas that have been in OSHA's domain for decades.

TSCA NCC's reading of the effect of Section 5(f)(5) does not suggest that Congress, in amending TSCA, intended to supplant OSHA's regulatory authority over workers exposed to any chemical substance that is "new" for TSCA purposes. For this reason, it would be prudent to minimize the likelihood that EPA's regulatory activities affecting occupational exposures to new chemicals may be construed to preempt OSHA's authority to regulate exposures of those same workers. TSCA NCC believes that a robust consultation process that assures that EPA does not unnecessarily encroach on OSHA regulation should suffice to prevent any unintended preemption. Furthermore, TSCA NCC believes that the existing 1991 MOU



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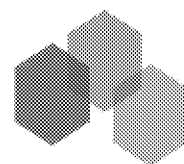
(and, as appropriate, the 1981 MOU) should be updated to clarify the effect of TSCA statutory changes, including orders and rules concerning workplace exposures for new chemical substances issued by EPA under new TSCA. Such a revision would also be an appropriate response by EPA to the directive in TSCA Section 26(l)(1) that EPA, within two years of enactment, develop any policies, procedures, and guidance that are determined to be necessary to carry out the amendments.

II. OSHA REGULATION OF OCCUPATIONAL EXPOSURE TO NEW CHEMICALS

OSHA has in place an extensive regulatory scheme, as well as enforcement mechanisms, governing chemical exposure in the workplace. OSHA's longstanding policy preference is to minimize workplace exposures to chemicals through engineering and process controls, which it may specify in substance-specific standards. In those circumstances where personal protective equipment (PPE) is needed to further limit worker exposure, OSHA has adopted PPE regulations; those for General Industry are found at 29 C.F.R. Part 1910, Subpart I. Section 1910.132 describes the current OSHA standards generally applicable to PPE and provides a framework for determining whether an employer has complied with those standards, while, as discussed below, respiratory protection specifically is addressed in Section 1910.134.

In a workplace inspection, OSHA's Certified Safety and Health Official (CSHO) makes the determination whether the employer has selected the particular PPE that is necessary to protect employees from identified hazards. An employer that fails to select adequate PPE generally is subject to a citation for violating 29 C.F.R. § 1910.132(d)(1)(i) unless a provision specific to the type of PPE involved applies instead. If an employer has not provided a written certification that a hazard assessment has been conducted, the inspector is directed to cite the employer for violating 29 C.F.R. § 1910.132(d)(2). If no specific PPE standard applies to the working conditions involved, or does not fully address a workplace hazard, the OSH Act's General Duty clause in Section 5(a) nonetheless requires the protection of the affected employees.

The OSH Act's General Duty clause requires every employer to furnish to each of its employees a workplace free from recognized hazards that cause, or are likely to cause, death or serious physical harm; it also requires every employer to comply with the occupational safety and health standards and all rules, regulations, and orders issued under the OSH Act. Thus, the General Duty clause adds a broad safety net and also underscores the workplace-centric nature of the OSH Act and of the intertwined responsibilities of both OSHA and individual employers in meeting specific occupational health and safety objectives. It is TSCA NCC's view that the



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General Duty clause requires employers to implement measures to prevent or to mitigate chemical exposures that may present a risk, including instances where the potential risk is identified as part of EPA's review of a new chemical substance and not fully addressed through OSHA's regulations.

OSHA also has issued detailed regulatory provisions addressing respiratory protection in the workplace; respiratory protection is disfavored as a matter of policy whenever engineering or process controls will suffice to limit occupational exposure. Respiratory protection in the form of PPE nonetheless is of particular importance for limiting chemical exposures, and is addressed both in 29 C.F.R. Subpart I at § 1910.134, as well as in various substance-specific 29 C.F.R. Part 1910 standards. The regulatory standard requires use of respirators where they are needed to protect employees from exposures to air contaminants above an exposure limit, or where they are otherwise necessary to protect employee health.

The standard places a range of responsibilities on employers as to the written respiratory protection program that must be in place, including procedures for respirator selection, use, fit, testing, cleaning, maintenance and repair; training in use and hazards; and medical evaluations of employees who use them, among other program elements. The employer is required to select and provide an appropriate respirator (National Institute for Occupational Safety and Health (NIOSH) certified) based on the respiratory hazard(s) present in the workplace, as well as workplace and user factors that affect respiratory performance and reliability. The assessment of workplace-specific hazards is a key prerequisite to the choice of the appropriate respirator; an employer who fails to assess those respiratory hazards and to select respiratory protection suitable for the purpose intended is subject to a citation for violating 29 C.F.R. § 1910.134(a)(2). Likewise, unless a substance-specific standard applies, an inspector can cite an employer for failing to provide the type of respirator needed for the substance and level of exposure involved as required under 29 C.F.R. § 1910.134(d).

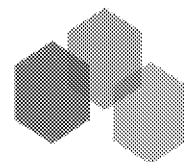
TSCA NCC's review of the relevant materials does not suggest that, in enacting new TSCA, Congress intended to alter the scope of the effect of these OSHA requirements. Absent any such indication, TSCA NCC believes that the OSHA regulatory structure, including but not limited to its approach to workplace- and employee-specific PPE requirements, continues to apply where a "new" chemical substance under TSCA is involved.

III. RECONCILING EPA'S OBLIGATION TO PROTECT AND EPA'S OBLIGATION TO CONSULT

Although EPA has an obligation to formulate and to adopt TSCA Section 5(e) orders that include measures to protect workers from exposure to new chemical substances, this duty only applies "to the extent necessary to protect against an unreasonable risk." When this duty is viewed in juxtaposition with the mandatory consultation requirement in new TSCA Section 5(f)(5), it is clear that EPA is required to evaluate the adequacy of the existing OSHA regulatory scheme, including the General Duty clause, and to adopt additional restrictions or prohibitions only when they are needed to protect against unreasonable risk.

Given the robust nature of the existing OSHA regulatory program, the proper role for EPA should be to provide hazard identification and risk assessment information that OSHA and affected employers can utilize in selecting appropriate PPE, including respiratory protection measures. For example, EPA can provide its hazard, exposure, and risk assessment information on a specific new chemical to OSHA and to the notifier, which will assist OSHA and affected employers in selecting the respiratory protection equipment and other PPE needed to comply with OSHA's regulations in 29 C.F.R. §§ 1910.134 and 1910.132. In TSCA NCC's view, when OSHA and the notifier receive EPA's hazard, exposure, and risk assessment for a new chemical substance, these materials must be considered by all employers who manufacture, process, distribute, or use the chemical in satisfying their obligation to provide a safe working environment. EPA could also make its new chemical hazard assessments more widely available, for example, by including them in its ChemView system. The chemical identity (where non-CBI), new chemical case number, and the accession number and generic name for CBI chemicals can also be included. In the case of commenced CBI new chemicals, EPA could make its appropriately sanitized hazard assessment available in responding to a *bona fide* request to ensure that future manufacturers are aware of its assessment. To ensure that this occurs, EPA could amend its *bona fide* procedures at 40 C.F.R. § 720.25 to include this step.

EPA can utilize specific restrictions in TSCA Section 5(e) consent orders to mitigate workplace exposure, but this authority is also less pervasive in nature than OSHA's broad authority to control occupational exposures. The same is true of EPA's use of Section 5(a)(2) significant new use rules (SNUR) to extend the requirements to entities beyond the notifier. Such approaches do not provide the same breadth of protection and the ongoing compliance responsibilities on the employer afforded by the OSH Act and OSHA's implementing measures. TSCA NCC believes that careful ongoing consultation with OSHA, as required under new TSCA, along with a full appreciation of the scope and effect of the OSH Act



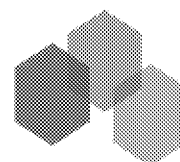
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and OSHA's implementing measures, is essential to ensure adequate protection of all workers while also assuring that EPA only adopts separate restrictions in consent orders to the "extent necessary" to protect against an unreasonable risk.

On balance, TSCA NCC believes that EPA should disfavor issuing TSCA Section 5(e) orders that mandate use of particular PPE or other workplace-specific measures to mitigate occupational exposure. Even when the measures in question merely replicate what the applicant itself has suggested in a proposed Safety Data Sheet (SDS), such prescriptive orders have a variety of significant disadvantages. Such orders ignore OSHA's established expertise and the robust existing regulatory program, risk creating disputes over whether the EPA action has preempted OSHA's general authority to protect the involved workers, will inevitably lead to conflicts with or disputes over interpretation of parallel OSHA requirements, and may have applicability that is significantly limited by jurisdictional factors. It merits noting, as well, that OSHA does not give its approval or sign-off to the recommendations contained in SDSs and that recommendations in Section 8 of an SDS as to PPE are by no means determinative from a compliance standpoint. OSHA relies as well on its own considerable expertise, on the degree to which any industry consensus standards may be relevant, and on the impact of site- or employee-specific circumstances. For all of these reasons, TSCA NCC also believes that it is of paramount importance that to meet its obligation under Section 5(f)(5), EPA promptly should create a mechanism for the necessary ongoing consultations with OSHA. TSCA NCC further recommends that EPA act swiftly to meet its responsibilities under Section 26(l)(1) and commence discussions with OSHA that will lead to an update of existing MOUs to delineate clearly each agency's role in regulating exposure to new chemical substances given the changes in new TSCA.

For the reasons elaborated above, TSCA NCC is of the view that for many, if not most, of the new chemicals for which EPA has proposed workplace restrictions under TSCA, the OSH Act and OSHA's regulatory program, once EPA has informed OSHA and the notifier of its occupational risk assessment, will be sufficient to ensure workplace protection and thereby make any unreasonable risk to workers "not likely." Section 5(e) requirements to restrict workplace exposures should be reserved for those instances where EPA has determined, after consultation with OSHA, that the OSH Act and OSHA's regulatory program are not sufficient to protect against unreasonable risk from workplace exposures and that TSCA action therefore meets the "extent necessary" requirement. To the extent that EPA proceeds as recommended by TSCA NCC and relies on the OSH Act and OSHA's regulatory program, this will also have the benefit of reducing EPA's administrative burden currently spent in negotiating consent orders and promulgating SNURs for occupational concerns. Such a change in approach could also allow



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NEW CHEMICALS
COALITION

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EPA to focus these regulatory resources on the potential risks to the environment and the general population -- areas that do not present the same level of overlapping authority and duplicative requirements as exist for workplace exposures.

Attachment

**OSHA**

English | Spanish

Find it in OSHA



A TO Z INDEX

ABOUT OSHA ▾ WORKERS ▾ EMPLOYERS ▾ REGULATIONS ▾ ENFORCEMENT ▾ TOPICS ▾ NEWS & PUBLICATIONS ▾ DATA ▾ TRAINING ▾

Memorandums of Understanding - Table of Contents

- **Information Date:** 01/19/1981
- **Agreement Agency:** The Office of Pesticides and Toxic Substances and U.S. Environmental Protection Agency

MEMORANDUM OF UNDERSTANDING
BETWEEN THE
OFFICE OF PESTICIDES AND TOXIC SUBSTANCES,
U.S. ENVIRONMENTAL PROTECTION AGENCY
AND THE
OCCUPATIONAL SAFETY AND HEALTH ADMINISTRATION,
U.S. DEPARTMENT OF LABOR
FOR

- GENERAL COOPERATION
- SHARING OF CONFIDENTIAL BUSINESS INFORMATION
- OSHA-EPA COOPERATION IN THE TSCA PREMANUFACTURE NOTIFICATION PROGRAM
- TRANSFER OF EPA INFORMATION ON SUBSTANTIAL RISK NOTICES

I. GENERAL WORKING AGREEMENT

This Memorandum of Understanding establishes a general working relationship between the Occupational Safety and Health Administration, U.S. Department of Labor, and the Office of Pesticides and Toxic Substances, U.S. Environmental Protection Agency, regarding matters having or potentially having an effect on the activities and responsibilities of the two agencies.

II. COORDINATION

To achieve the coordination desired by both EPA AND OSHA, each agency hereby designates a coordinating office. The coordinating office for the Office of Pesticides and Toxic Substances (OPTS) will be the Office of Toxics Integration (OTI); for the Occupational Safety and Health Administration (OSHA), the Division of Interagency Programs. These offices shall serve as the initial communication link between the two agencies. Future specific agreements will be made by the program offices of OSHA and EPA's Office of Toxic Substances. Parts A, B and C below are directed at specific areas of coordination for sharing of confidential business information, OSHA's cooperation in the OPTS premanufacture notification program, and referral to OSHA of TSCA section 8(e) notices.

In carrying out their respective responsibilities, OPTS and OSHA will, to the extent practicable, consult and exchange information with each other through the coordinating offices. Specifically they will:

- (1) Coordinate programs, including the development of standards, to avoid duplication of effort, to assist in setting priorities, and share information and research;
- (2) When appropriate, consider the development of joint regulatory efforts. If no joint efforts are possible, both agencies will coordinate the development of any regulations concerning occupational exposure to new chemicals, to the extent feasible;
- (3) Exchange information and report on general enforcement matters and on particular situations of common concern to each agency;
- (4) Make every effort to achieve uniformity of approach in long-range planning;
- (5) Obtain legal and policy positions on statutory authority regarding the extent to which the other agency can remedy a particular condition or matter that may be within the regulatory purview of the agencies;
- (6) Use communication systems available to both agencies for educational services to the public about safety and health topics.

A. Confidential Business Information Exchange**PURPOSE:**

This section allows OSHA to have access to confidential business information (CBI) submitted to EPA under the Toxic Substances Control Act (TSCA). OSHA will use this information to assist in fulfilling its duty to protect worker health under the Occupational Safety and Health Act of 1970.

SCOPE:

OSHA is permitted access to all confidential business information submitted to EPA under TSCA. When OSHA requests transfer of specific CBI, a justification of the need for access will be submitted through the OSHA Document Control Office (DCO) to any DCO in OPTS. OSHA will treat all such information in accordance with the Memorandum of Understanding. When OPTS initiates the transfer of CBI, a justification of OSHA's need for access should be prepared by the appropriate EPA program official and submitted to an OPTS DCO prior to the transfer of any documents containing confidential business information. The appropriate OPTS DCO must approve the justification prior to transfer of CBI.

PROVISIONS:

- (1) OSHA will protect information received from EPA under this agreement by following the procedures set forth in its "OSHA TSCA Confidential Business Information Security Manual." The procedures have been approved by EPA's Inspector General's Office, and they meet or exceed the requirements of EPA's own "TSCA Confidential Business Information Security Manual."
- (2) OSHA agrees that it will not release or transfer TSCA confidential business information outside of OSHA without the prior approval of EPA.
- (3) OSHA will normally return confidential documents to EPA within one year, but with approval by the OPTS Document Control Officer, will be granted extensions. In addition, with approval of the OPTS Document Control Officer, OSHA may destroy the documents according to the requirements of the EPA TSCA Security Manual instead of returning them to EPA.

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(4) OSHA personnel will be made aware of the possible criminal liabilities that may result from unauthorized release of CBI and will sign the TSCA-Federal non-EPA employee confidentiality agreement (Appendix 14).

(5) The Information Control Branch of the Management Support Division of OPTS (EPA) will provide initial CBI training to appropriate OSHA staff.

(6) A physical inspection of OSHA's security facilities will be made by EPA. No exchange of TSCA CBI will be made until such facilities are found to be satisfactory. There will be future periodic inspections of OSHA's security program by EPA.

(7) Following inspection and approval of OSHA's security facilities, a Federal Register notice will be published announcing this agreement and will provide the required ten days of notice, covering all future sharing of data under this section, pursuant to section 2.209 of EPA's regulations on confidentiality of business information, 40 CFR Part 2, Subpart B.

B. Premanufacture Notification (PMN) Data Exchange Procedure

PURPOSE:

This section deals with the exchange of PMN data between OSHA and OPTS. PMNs can provide information about possible worker exposure to new chemicals before they are produced on a large scale, enabling both OSHA and OPTS to discuss any possible hazards to exposed worker populations and, if necessary, coordinate action on these chemicals.

SCOPE:

OSHA and OPTS will work to assure that complete and timely notification is made concerning PMNs which may involve or affect occupational exposures to chemical hazards, and also to assure necessary coordination between OPTS and OSHA, including joint review of selected PMNs. This will permit OPTS to have the benefit of OSHA expertise in assessing occupational exposure risks, and will alert OSHA to possible chemical threats to worker health.

PROVISIONS:

To assure the above conditions are met, the following procedures are established:

(1) Contacts with OSHA concerning PMNs will be initiated by the Notice Manager for a particular PMN or by OTI through the designated individual in the OSHA Division of Interagency Programs. This individual will receive all data and information from EPA and be responsible for the response from OSHA. This individual shall coordinate the OSHA response or refer the EPA Notice Manager to the appropriate OSHA staff.

(2a) In order to assure that OSHA is informed of the status of EPA actions on PMNs, EPA's Chemical Control Division (CCD) will forward to the OSHA representative, as available, a copy of EPA's weekly PMN report. OSHA will use the report to identify PMNs of potential concern about which OSHA has not been contacted by OTI or the Notice Manager.

(2b) If the occupational exposure to a chemical is a concern during initial review, the Notice Manager or OTI will notify OSHA. EPA may request OSHA data concerning the chemical or its analogue or may refer the PMN to OSHA for information or consideration if no TSCA action is to be taken.

(2c) If a PMN for which there is concern regarding potential occupational exposure, goes into a more detailed review, the Notice Manager or OTI will notify OSHA. During the detailed review, Chemical Control Division may request technical assistance from OSHA to aid in EPA's assessment of the PMN and invite OSHA to participate in the work group.

(3) During the development of any regulatory action on a PMN for which occupational exposure is of concern, CCD will consult with OSHA. OSHA may be asked to participate in the detailed review work group for the PMN to assist in development of regulatory options. At that time, OPTS will provide OSHA with copies of documents generated by the OPTS initial review which describe the problem. As a member of this group the OSHA staff may be involved in reviewing draft regulatory actions and will be provided with a copy of the package which enters the EPA official rulemaking and clearance process.

(4) EPA will notify OSHA representatives of the final action taken by EPA on any PMN where occupational exposure is a concern.

C. Notices of Substantial Risk

PURPOSE:

This section provides a mechanism for EPA to provide OSHA with information submitted by industry under section 8(e) of TSCA, Notices of Substantial Risk.

SCOPE:

Section 8(e) of TSCA requires that any person who manufactures, processes, or distributes a chemical substance or mixture and who obtains information which reasonably supports the conclusion that the substance or mixture presents a substantial risk of injury to health or the environment shall immediately inform EPA.

For each 8(e) notice received, the OPTS Chemical Hazard Information Branch (CHIB) prepares a status report. CHIB will, by this agreement, refer to OSHA any 8(e) notices in connection with which CHIB identifies an occupational exposure of concern. OTI will coordinate any necessary follow-up work with OSHA, such as plans for further evaluation or discussion of regulatory action.

III. AUTHORITY

The Office of Pesticides and Toxic Substances enters into this agreement under the authority of Sections 9 and 14 of the Toxic Substances Control Act (15 USC 2601, et seq.). Section 9 of TSCA requires certain coordination of actions taken under TSCA with actions taken under other Federal laws. Section 14 of TSCA provides that confidential business information may be disclosed to any officer or employee of the United States in connection with the official duties of such officer or employee under any law for the protection of health or the environment.

The Occupational Safety and Health Administration enters into this agreement under authority of the Occupational Safety and Health Act of 1970 (29 USC 651, et seq.), Section 7(c)(1). That section allows the Secretary of Labor to "use, with the consent of any Federal agency, the services, facilities, and personnel of such agency, with or without reimbursement...."

IV. PERIOD OF AGREEMENT

This Memorandum of Understanding shall continue in effect unless modified by mutual assent of the parties or terminated by either party upon a 30-day advance written notice to the other party.

This Memorandum does not preclude the parties from entering into separate agreements setting forth procedures for special programs which can be handled more efficiently and expeditiously by such special agreement.

Nothing in this agreement is intended to diminish or otherwise affect the authority of either agency to carry out its respective statutory functions.

This Memorandum will become effective on the date of the last signature.

Marilyn C. Bracken
Associate Assistant Administrator
for Toxics Integration

Warren R. Muir
Deputy Assistant Administrator
for Toxics Substances

Appointment

From: Fehrenbacher, Cathy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=369151285d0143bba4f6fb3f9991e583-CFehrenb]
Sent: 10/6/2020 6:34:08 PM
To: Franz, Christina [Christina_Franz@americanchemistry.com]

Subject: Accepted: TSCA Section 5 EPA Meeting

Location: **Ex. 6 Conference Code**

Start: 10/8/2020 6:30:00 PM

End: 10/8/2020 7:30:00 PM

Show Time As: Busy

12

Appointment

From: Henry, Tala [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=8BFC0A617A4A43BAA8856541C70622BE-THENRY02]
Sent: 12/14/2018 2:59:03 PM
To: Renee Lani [renee_lani@americanchemistry.com]
Subject: Accepted: FW: Technical Meeting with TSCA Section 5 Testing Consortium and ScitoVation
Location: 700 2nd Street NE, 20002
Start: 12/13/2018 2:00:00 PM
End: 12/13/2018 5:00:00 PM
Show Time As: Busy

Appointment

From: Renee Lani [renee_lani@americanchemistry.com]
Sent: 10/24/2018 12:18:47 PM
To: Renee Lani [renee_lani@americanchemistry.com]; Henry, Tala [Henry.Tala@epa.gov]; Scarano, Louis [Scarano.Louis@epa.gov]; Lowit, Anna [Lowit.Anna@epa.gov]; Camacho, Iris [Camacho.Iris@epa.gov]; Irwin, William [Irwin.William@epa.gov]; Schweer, Greg [Schweer.Greg@epa.gov]; Lloyd, Matthew [Lloyd.Matthew@epa.gov]
Subject: Technical Meeting with TSCA Section 5 Testing Consortium and ScitoVation
Location: 700 2nd Street NE, 20002
Start: 12/13/2018 2:00:00 PM
End: 12/13/2018 5:00:00 PM
Show Time As: Busy

Appointment

From: Renee Lani [renee_lani@americanchemistry.com]
Sent: 10/24/2018 12:18:47 PM
To: Renee Lani [renee_lani@americanchemistry.com]; Henry, Tala [Henry.Tala@epa.gov]; Scarano, Louis [Scarano.Louis@epa.gov]; Lowit, Anna [Lowit.Anna@epa.gov]; Camacho, Iris [Camacho.Iris@epa.gov]; Irwin, William [Irwin.William@epa.gov]; Schweer, Greg [Schweer.Greg@epa.gov]; Lloyd, Matthew [Lloyd.Matthew@epa.gov]
Subject: FW: Technical Meeting with TSCA Section 5 Testing Consortium and ScitoVation
Location: 700 2nd Street NE, 20002
Start: 12/13/2018 2:00:00 PM
End: 12/13/2018 5:00:00 PM
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Sent: 10/24/2018 12:18:47 PM
To: Renee Lani [renee_lani@americanchemistry.com]; Henry, Tala [Henry.Tala@epa.gov]; Scarano, Louis [Scarano.Louis@epa.gov]; Lowit, Anna [Lowit.Anna@epa.gov]; Camacho, Iris [Camacho.Iris@epa.gov]; Irwin, William [Irwin.William@epa.gov]; Schweer, Greg [Schweer.Greg@epa.gov]; Lloyd, Matthew [Lloyd.Matthew@epa.gov]
Subject: Technical Meeting with TSCA Section 5 Testing Consortium and ScitoVation
Location: 700 2nd Street NE, 20002
Start: 12/13/2018 2:00:00 PM
End: 12/13/2018 5:00:00 PM
Show Time As: Busy

FYI

-----Original Appointment-----

From: Lani, Renee <renee_lani@americanchemistry.com>
Sent: Tuesday, October 23, 2018 5:20 PM
To: Lani, Renee; Lloyd, Matthew
Subject: Technical Meeting with TSCA Section 5 Testing Consortium and ScitoVation
When: Thursday, December 13, 2018 9:00 AM-12:00 PM (UTC-05:00) Eastern Time (US & Canada).
Where: 700 2nd Street NE, 20002

Agenda to follow.

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Appointment

From: Clark, Sharon [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=6821D9DDE270456CAA67A7114E49F707-CLARK, SHARON]
Sent: 3/6/2019 9:43:40 PM
To: Henry, Tala [Henry.Tala@epa.gov]; Moss, Kenneth [Moss.Kenneth@epa.gov]; Ryan Schmit (schmit.ryan@epa.gov) [schmit.ryan@epa.gov]; Pierce, Alison (Pierce.Alison@epa.gov) [Pierce.Alison@epa.gov]; Franz, Christina [Christina_Franz@americanchemistry.com]
CC: Schweer, Greg [Schweer.Greg@epa.gov]; Osman-Sypher, Sahar [Sahar_Osman-Sypher@americanchemistry.com]; Vendinello, Lynn [Vendinello.Lynn@epa.gov]; Mark Duvall [MDuvall@bdlaw.com]; Lisa Marie Nespoli [lisamarie.nespoli@covestro.com]; Mark Joseph McKinney [mark.mckinney@basf.com]; Marcia Levinson [marcia.levinson@covestro.com]; Stedeford, Todd [Stedeford.Todd@epa.gov]; Adam Kuhl [adam_kuhl@huntsman.com]; Wormell, Lance [Wormell.Lance@epa.gov]; Arnold, Scott (M) [SMArnold@dow.com]; Cynthia Graham [cynthia_graham@huntsman.com]
BCC: DCRoomEast3371A/DC-EPA-EAST-OCSPP-OPPT [DCRoomEast3371A@epa.gov]
Subject: Meeting with ACC
Attachments: ACC letter to EPA re April 10 meeting on isocyanates - 4-5-19.pdf; ACC_EPA Meeting Agenda on Isocyanates-Based SNURs 2019 04 10.pdf
Location: DCRoomEast3371A/DC-EPA-EAST-OCSPP-OPPT
Start: 4/10/2019 12:00:00 PM
End: 4/10/2019 1:00:00 PM
Show Time As: Busy

Teleconference Line:

Ex. 6 Personal Privacy (PP) - conference code/call in number

Conference ID: Ex. 6 Personal Privacy (PP) - conference code/call in number

LEADER: Tala

Meeting Location:

Environmental Protection Agency
William Jefferson Clinton East Building
1201 Constitution Avenue NW
Conference Room: 3371A (3rd Floor)
Washington, DC 20460

Please bring photo identification and once through security, please have them call (202) 564-3810 for an escort to meeting location.

Please provide list of names of five or more attendees coming from your group – this will speed up clearing security check-in.



April 5, 2019

By email to henry.tala@epa.gov

Dr. Tala Henry (74013T)
Acting Deputy Director for Programs
Office of Pollution Prevention and Toxics
Office of Chemical Safety and Pollution Prevention
Environmental Protection Agency
1200 Pennsylvania Avenue, N.W.
Washington, DC 20460

Re: EPA's Approach to Isocyanate-Based Polymers Under TSCA Section 5

Dear Dr. Henry:

On behalf of the American Chemistry Council and its Diisocyanates and Aliphatic Diisocyanates Panels (collectively ACC), thank you for scheduling a meeting with us on April 10 at 8:00 a.m. This letter presents the questions ACC would like to discuss at the meeting.

The purpose of the meeting is to help ACC better understand EPA's approach to isocyanate-based polymers under section 5 of TSCA. The outcome of the meeting could help ACC and its members, and the customers of those members, develop polymers with improved risk profiles, and also help expedite the SNUR rulemaking process.

As a preliminary matter, we point out that EPA published an Action Plan for methylene diphenyl diisocyanate (MDI) and related compounds in April 2011. Since then, it has issued final or proposed SNURs for at least 58 isocyanate-based polymers and prepolymers. Of those 58 SNURs, EPA has finalized 28. A total of 30 SNURs, more than half, remain under review. EPA last finalized a SNUR for an isocyanate-based polymer in December 2014, over four years ago. See Attachment 1 for details.

ACC has submitted comments on almost all of the 30 proposed SNURs raising concerns that – to date – EPA has not answered. We hope that the meeting will address those concerns.

1. When will EPA update the Chemical Categories description for diisocyanates?

ACC would like to know when EPA will update at least the Chemical Categories description on diisocyanates. EPA has told ACC that it no longer follows the guidance in that description. ACC members and their customers rely on EPA guidance as to what limits are acceptable, so an updated description is critical to ensuring our members have clear guidance regarding the parameters EPA considers when issuing these SNURs.

2. Why does EPA propose maximum percentages for residual isocyanates in some section 5(e) orders and SNURs for isocyanate-based polymers, and not in others, and use differing percentages when it does propose these limitations?

In several recent SNURs for isocyanate-based polymers, EPA has proposed a ceiling on residual isocyanate content. ACC would also like to understand what EPA means by “residual isocyanate.” Does this refer only to unreacted monomer? It is important for EPA to clarify this term, because EPA does not have authority under section 5 to regulate the monomer itself as it is used as a reactant (an existing use), and the monomer is not part of the polymer or pre-polymer that is the PMN substance.

The proposed SNURs with a ceiling include those in the following PMN substances:

- P-18-51: “It is a significant new use to import the substance with greater than 0.1% isocyanate content.”
- P-17-374: “It is a significant new use to import the substance with more than 0.1% residual isocyanate.”
- P-17-361: “It is a significant new use to manufacture the substance containing greater than 0.25% residual isocyanate”
- P-17-304: “It is a significant new use to manufacture (includes importing) the substance to contain more than 0.1% residual isocyanate by weight.”
- P-17-222: “It is a significant new use to import the chemical substance containing greater than 0.15 percent residual isocyanate.”
- P-17-170: “It is a significant new use to manufacture the chemical substance containing greater than 0.1 percent residual isocyanate”
- P-17-10: “It is a significant new use to manufacture the chemical substance ... containing greater than 0.1% residual isocyanate.”
- P-16-493: “It is a significant new use to import the PMN substance to contain more than 0.1% residual isocyanate by weight.”
- P-16-363: “It is a significant new use to manufacture, process, or use the substance with a residual of free isocyanate monomers greater than 0.1 percent by weight.”
- P-16-99: “It is a significant new use to manufacture the chemical substance containing greater than 0.2% residual isocyanate.”
- P-15-707: “A significant new use is any manufacture, processing, or use of the PMN substance with more than [0.1]% residual isocyanate by weight.”¹

¹ The proposed regulatory text refers to “1%”, but the preamble refers to “0.1%”. ACC assumes that EPA meant to refer to “0.1%” in the regulatory text.

- P-15-706: “A significant new use is any manufacture, processing, or use of the PMN substance with more than 0.1% residual isocyanate by weight.”

Thus, EPA has proposed a maximum percentage limitation in 12 of the 58 SNURs for isocyanate-based polymers since April 2011. It proposed a 0.1% limit (variously phrased) for 10 of them. The other percentages were 0.15%, 0.2%, and 0.25%.

ACC would like to understand why EPA proposed a maximum percentage limitation in some section 5(e) orders and SNURs for isocyanate-based polymers and not in others, and why it selected 0.1% in most cases, but proposed higher percentages in three instances.

3. Why does EPA propose limits on average molecular weight in some section 5(e) orders and SNURs for isocyanate-based polymers, and not in others, and use differing molecular weight limits when it does propose them?

In several recent SNURs for isocyanate-based polymers, EPA has proposed a ceiling on low-molecular weight species. They include proposed SNURs for the following PMN substances:

- P-18-40: “It is a significant new use to import the substance if the number average molecular weight is less than or equal to 1000 daltons.”
- P-17-374: “It is a significant new use to import the substance at a number average molecular weight less than 1000 daltons.”
- P-17-361: “It is a significant new use to manufacture the substance ... [with] an average molecular weight less than 2,280 daltons.”
- P-17-170: “It is a significant new use to manufacture the chemical substance ... [with] an average molecular weight below 1,000 daltons.”
- P-17-10: “It is a significant new use to manufacture the chemical substance with an average molecular weight below 2,000 daltons”
- P-15-559: “A significant new use of the substance is manufacture of the substance where the average molecular weight is below 7,500 daltons, and where any molecular weight species is below 1,000 daltons.”
- P-15-278: “The significant new use is manufacture of the substance where the average molecular weight is below 2,500 daltons and where any molecular weight species is below 1,000 daltons.”
- P-12-326: “The significant new uses are: (i) Industrial, commercial, and consumer activities. Requirements as specified in § 721.80(j) (manufacture, processing, or use where the molecular weight is 1000 daltons or more).”² [Note: presumably should be 1000 daltons or less.]

Thus, EPA proposed a minimum molecular weight in 8 of the 58 SNURs since April 2011. The minimums proposed have included 1,000 daltons, 2,000 daltons, 2,500 daltons, and 2,280 daltons. In addition, EPA has expressed concerns about average molecular weights below 7,500 daltons.

² The text should probably refer to “1000 daltons or less,” not “1000 daltons or more.” The preamble refers to “1000 daltons or less.”

Some of these proposed minimums and the statement of concern are inconsistent with the 2010 Chemical Categories section on diisocyanates. It states:

Structures with an isocyanate equivalent weight of $\geq 5,000$ are presumed not to pose a hazard under any condition. Typically, concerns are confined to those species with molecular weights $< 1,000$.

ACC would like to understand why EPA proposed a minimum average molecular weight in some section 5(e) orders and SNURs for isocyanate-based polymers and not others, and why it selected 1,000 daltons in most cases, but proposed higher molecular weights in three cases.

4. How Did EPA Calculate the NCEL of 0.9 mg/m³?

For 3 of the 58 SNURs for isocyanate-based polymers since April 2011, EPA has proposed a New Chemical Exposure Limit (NCEL) of 0.9 mg/m³. They are the proposed SNURs for P-16-99, P-15-707, and P-15-706.

The SNUR for P-04-834 set a NCEL of 0.05 mg/m³ TWA₈. In addition, the section 5(e) order for P-16-493 cautioned:

Inhalation exposure should be limited to < 0.05 mg/m³ as an 8-hours time-weighted average (TWA) for combined polyisocyanates and diisocyanates.

ACC would like to understand the basis for these NCELs and how EPA calculated the 0.9 mg/m³ NCEL and the 0.05 mg/m³ NCEL.

5. Which health effects does EPA associate with isocyanates?

As indicated in the attached table, EPA has described its expected health effects from isocyanate-based polymers in widely varying terms. It mentioned dermal and respiratory sensitization consistently. In some section 5(e) orders and SNURs, however, EPA has asserted concerns for oncogenicity, mutagenicity, neurotoxicity, developmental toxicity, and lung toxicity based on cationic binding. Most, however, have not referred to those health effects.

ACC would like to understand which health effects of isocyanates are of concern to EPA; why it cites some health effects for some substances and not others; and whether the isocyanate-based concerns vary from substance to substance and, if so, what the basis is for EPA's selection of particular health effects for a given SNUR.

6. Has EPA reviewed the basis for its assertion that isocyanates are the leading cause of occupational asthma, with an incidence rate as high as 20%?

The section 5(f) order for P-17-24 and P-17-25 declared:

Isocyanate exposure has been identified as the leading attributable cause of work-related asthma, and prevalence in the exposed workforce has been estimated at 1-20 percent (see Refs. 1 and 2).

This statement is taken verbatim from the proposed SNUR for toluene diisocyanates and related compounds, 80 Fed. Reg. 2068, 2070 (Jan. 15, 2015). Numerous section 5(e) orders do not make this statement explicitly, but instead they refer to the 2006 NIOSH Alert, “Preventing Asthma and Death from MDI Exposure During Spray-on Truck Bed Liner and Related Applications,” which states:

Isocyanates are the leading attributable chemical cause of occupational asthma in the United States and many other industrialized countries [Tarlo et al. 1997b].

ACC has reviewed the sources cited by EPA and NIOSH for this assertion and found them to be outdated and inaccurate. See Attachment 2.

ACC encourages EPA to review the more recent scientific literature, which does not support the assertion that diisocyanates are the leading cause of occupational asthma. Recent data show a consistent picture of a decline in asthma rates associated with diisocyanates over the last decade, even as production rates of diisocyanates have increased. The reduction in diisocyanate-related occupational asthma is primarily due to a variety of product stewardship activities, including education and training, enhanced worker awareness, improved work practices, use of less volatile diisocyanate forms (e.g., pre-polymers), improved engineering controls (e.g., containment and/or ventilation), better medical surveillance programs, minimization of peak exposures, and continuing emphasis on compliance with existing exposure standards. These product stewardship efforts are key to further reductions in cases.

According to the NIOSH Work-Related Lung Disease Surveillance System (eWoRLD), in the four U.S. states surveyed (California, Massachusetts, Michigan, and New York) the most recent work-related asthma statistics from 2009-2012 indicate that diisocyanates are not in the top ten “frequently reported agent categories associated with work-related asthma,” falling to number 19 (1.0% of work-related asthma cases).

7. Why is EPA not finalizing SNURs for isocyanate-based polymers?

EPA currently has 30 proposed SNURs for isocyanate-based polymers pending review. It has not finalized a proposed SNUR or a direct final SNUR for an isocyanate-based polymer since December 2014, over four years ago.

Any manufacturers and processors of these polymers, other than the PMN submitters, should be subject to the same restrictions as those to which the PMN submitters are subject.

ACC would like to understand why EPA continues to issue direct final SNURs and/or proposed SNURs for isocyanate-based polymers, but does not finalize them. ACC also requests a response to the comments it has submitted on many of the pending SNURs.

8. What is the significance of the medical surveillance requirement in a recent section 5(e) order and proposed SNUR?

For the first time that ACC is aware, EPA has included a medical surveillance requirement in a section 5(e) order and proposed SNUR. The proposed SNUR for P-17-231 states:

It is a significant new use to manufacture the substance without conducting medical surveillance as specified in the Order.

It is not clear whether the medical surveillance requirement was prompted by EPA concerns for isocyanates or for other moieties in that polymer.

ACC would like to understand why EPA included a medical surveillance requirement in that section 5(e) order and proposed SNUR (and, in particular, whether it was prompted by a concern about isocyanates). ACC would also like to know whether EPA intends to require medical surveillance in future section 5(e) orders and SNURs for isocyanate-based polymers, and, if so, what the basis is for this requirement and the intended scope of that medical surveillance.

Moving forward, ACC would very much like to partner with EPA to resolve the issues outlined above. ACC is available to educate Agency staff on our chemistries to help expedite chemical reviews and address EPA's concerns. In addition, we would like to work with EPA scientists to design test plans to develop toxicological data to fulfill apparent data gaps, particularly for isocyanate prepolymers.

We look forward to the opportunity to discuss these suggestions with your staff at the April 10 meeting. In the meantime, if you have questions or comments, please contact Christina Franz at (202) 249-6406 and christina_franz@americanchemistry.com, or Sahar Osman-Sypher at (202) 249-6721 and sahar_osman-sypher@americanchemistry.com.

Sincerely,

Ex. 6 Personal Privacy (PP)

Sahar Osman-Sypher
Director, Diisocyanates and Aliphatic
Diisocyanates Panels

Ex. 6 Personal Privacy (PP)

Christina Franz
Senior Director, Regulatory & Technical
Affairs

Attachments

cc: Alexandra Dunn
Dr. Jeffery Morris

Attachment 1

SNURs for Isocyanate-Based Polymers Since April 2011

PMN No.	C.F.R. Citation / F.R. Citation	Status of SNUR	Preamble Statement re Isocyanates	New Chemical Exposure Limit (NCEL)?	Special Restrictions in SNUR?
P-18-51	Proposed 40 C.F.R. § 721.11242 84 Fed. Reg. 9999 (Mar. 19, 2019) (proposed SNUR)	Comment period closes May 3, 2019.	§ 5(e) order: None specifically identified as attributable to isocyanates.	No	“It is a significant new use to import the substance with greater than 0.1% isocyanate content.”
P-18-40	Proposed 40 C.F.R. § 721.11239 84 Fed. Reg. 9999 (Mar. 19, 2019) (proposed SNUR)	Comment period closes May 3, 2019.	§ 5(e) order: None specifically identified as attributable to isocyanates.	No	“It is a significant new use to import the substance if the number average molecular weight is less than or equal to 1000 daltons.”

PMN No.	C.F.R. Citation / F.R. Citation	Status of SNUR	Preamble Statement re Isocyanates	New Chemical Exposure Limit (NCEL)?	Special Restrictions in SNUR?
P-17-374	Proposed 40 C.F.R. § 721.11239 84 Fed. Reg. 9999 (Mar. 19, 2019) (proposed SNUR)	Comment period closes May 3, 2019.	§ 5(e) order unavailable. None specifically identified in SNUR preamble as attributable to isocyanates.	No	“It is a significant new use to import the substance with more than 0.1% residual isocyanate. It is a significant new use to import the substance at a number average molecular weight less than 1000 daltons.”
P-17-361	Proposed 40 C.F.R. § 721.11211 83 Fed. Reg. 57634 (Nov. 15, 2018) (proposed SNUR)	Under EPA review. Comment period closed Dec. 31, 2018.	§ 5(e) order: “The concern for eye and skin irritation, and sensitization (dermal and respiratory) is based primarily on the isocyanate moiety.”	No	“It is a significant new use to manufacture the substance containing greater than 0.25% residual isocyanate or an average molecular weight less than 2,280 daltons.”

PMN No.	C.F.R. Citation / F.R. Citation	Status of SNUR	Preamble Statement re Isocyanates	New Chemical Exposure Limit (NCEL)?	Special Restrictions in SNUR?
P-17-304	Proposed 40 C.F.R. § 721.11206 83 Fed. Reg. 57634 (Nov. 15, 2018) (proposed SNUR)	Under EPA review. Comment period closed Dec. 12, 2018.	§ 5(e) order: “Toxicological Endpoints of Concern: There is concern for sensitization EPA's estimate of the human health hazard of the PMN substance is based on its estimated physical/chemical properties and other structural information, including the presence of Low Molecular Weight (LMW) moieties in the polymer.”	No	“It is a significant new use to manufacture (includes importing) the substance to contain more than 0.1% residual isocyanate by weight.”
P-17-231	Proposed 40 C.F.R. § 721.11112 83 Fed. Reg. 43538 (Aug. 27, 2018) (direct final SNUR); 83 Fed. Reg. 43607 (Aug. 27, 2019) (proposed SNUR)	Direct final SNUR withdrawn Oct. 24, 2018. Proposed SNUR under review.	§ 5(e) order: “No significant health concerns for the PMN substance as it is now. If made differently (different ratio of monomers), there may be isocyanate moieties present that would be of concern for human health endpoints of dermal sensitization, respiratory sensitization, lung effects, neurotoxicity and developmental toxicity. Basis: Diisocyanate Chemicals Category NIOSH Alert on Preventing Asthma and Death from MDI Exposure During Spray-on Truck Bed Liner and Related Applications As the PMN substance is currently described, there is no risk to workers, but if the substance is made with a different percent residual of isocyanate, there may be unreasonable risk to workers.”	No	“It is a significant new use to manufacture the substance without conducting medical surveillance as specified in the Order.”

PMN No.	C.F.R. Citation / F.R. Citation	Status of SNUR	Preamble Statement re Isocyanates	New Chemical Exposure Limit (NCEL)?	Special Restrictions in SNUR?
P-17-222	Proposed 40 C.F.R. § 721.11111 83 Fed. Reg. 43538 (Aug. 27, 2018) (direct final SNUR); 83 Fed. Reg. 43607 (Aug. 27, 2019) (proposed SNUR)	Direct final SNUR withdrawn Oct. 24, 2018. Proposed SNUR under review.	§ 5(e) order: “There are concerns for dermal sensitization, respiratory sensitization, lung effects, neurotoxicity and developmental toxicity. Basis: Diisocyanate Chemicals Category NIOSH Alert on Preventing Asthma and Death from MDI Exposure During Spray-on Truck Bed Liner and Related Applications As manufactured, there are no risks to workers for exposure of the PMN. Due to the possibility of isocyanate residuals if it is made different, there is concern for sensitization. Exposure to diisocyanate may cause the following effects: skin irritation and allergic reaction, respiratory irritation, respiratory sensitization, and lung toxicity; some diisocyanates also may cause cancer. It is especially important to note that contact with the skin may lead to respiratory sensitization or cause other allergic reactions. Workers should take precaution to avoid breathing vapors, mists or aerosols. Inhalation exposure should be limited to <0.05 mg/m ³ as an 8-hours time-weighted average (TWA) for combined polyisocyanates and diisocyanates.”	No	“It is a significant new use to import the chemical substance containing greater than 0.15 percent residual isocyanate.”

PMN No.	C.F.R. Citation / F.R. Citation	Status of SNUR	Preamble Statement re Isocyanates	New Chemical Exposure Limit (NCEL)?	Special Restrictions in SNUR?
P-17-170	Proposed 40 C.F.R. § 721.11107 83 Fed. Reg. 43538 (Aug. 27, 2018) (direct final SNUR); 83 Fed. Reg. 43607 (Aug. 27, 2019) (proposed SNUR)	Direct final SNUR withdrawn Oct. 24, 2018. Proposed SNUR under review.	§ 5(e) order: “Further, if the polymer were made differently there could be other hazards. For example, if made differently, there could be free isocyanates. See [Diisocyanate Chemicals Category ...]	No	“It is a significant new use to manufacture the chemical substance containing greater than 0.1 percent residual isocyanate or an average molecular weight below 1,000 daltons.”

P-17-24	<p>Proposed 40 C.F.R § 721.11159</p> <p>83 Fed. Reg. 49806 (Oct. 3, 2018) (direct final SNUR); 83 Fed. Reg. 49903 (Oct. 3, 2019) (proposed SNUR)</p>	<p>Direct final SNUR withdrawn Dec. 4, 2018. Proposed SNUR under review.</p>	<p>§ 5(f) order: “Respiratory and dermal sensitization and lung and mucous membrane irritation based on the isocyanate moiety Diisocyanates are well-known dermal, eye, and inhalation irritants and sensitizers based on worker data. They have been documented in the workplace to cause asthma and respiratory problems, such as hypersensitivity pneumonitis, an inflammation of the lungs. In severe cases, there have been reported fatal reactions Isocyanate exposure has been identified as the leading attributable cause of work-related asthma, and prevalence in the exposed workforce has been estimated at 1-20 percent (see Refs. 1 and 2). Once a worker is sensitized to diisocyanates, subsequent exposures can trigger severe asthma attacks. Spray application and heated processes are associated with higher incidences of asthma than other application methods because they can generate airborne isocyanate vapors and mists, which lead to worker exposure via the respiratory and dermal routes. Most workers who develop diisocyanate asthma have experienced long periods of exposure (months or longer); however, the minimum exposure to isocyanates that can elicit sensitization responses or asthma is unknown. In addition, immune response and subsequent disease in humans can vary significantly</p>	No	No
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PMN No.	C.F.R. Citation / F.R. Citation	Status of SNUR	Preamble Statement re Isocyanates	New Chemical Exposure Limit (NCEL)?	Special Restrictions in SNUR?
			between individuals (Ref. 3). Fatalities linked to diisocyanate exposures in sensitized persons have been reported (Refs. 4 and 5.) More information on isocyanate effects, could be found in the Diisocyanate Chemicals Category ...; or in the publication from Department of Health and Human Services, Center for Disease Control 'NIOSH Alert on Preventing Asthma and Death from MDI Exposure During Spray-on Truck Bed Liner and Related Applications'"		
P-17-25	Proposed 40 C.F.R. § 721.11160 83 Fed. Reg. 49806 (Oct. 3, 2018) (direct final SNUR); 83 Fed. Reg. 49903 (Oct. 3, 2019) (proposed SNUR)	Direct final SNUR withdrawn Dec. 14, 2018. Proposed SNUR under review.	Same as above	No	No

PMN No.	C.F.R. Citation / F.R. Citation	Status of SNUR	Preamble Statement re Isocyanates	New Chemical Exposure Limit (NCEL)?	Special Restrictions in SNUR?
P-17-10	Proposed 40 C.F.R. § 721.11084 83 Fed. Reg. 40986 (Aug. 17, 2018) (direct final SNUR); 83 Fed. Reg. 41039 (Aug. 17, 2018) (proposed SNUR)	Direct final SNUR withdrawn Oct. 11, 2018. Proposed SNUR under review.	§ 5(e) order: None specifically identified as attributable to isocyanates.	No	“It is a significant new use to manufacture the chemical substance with an average molecular weight below 2,000 daltons or containing greater than 0.1% residual isocyanate.”

PMN No.	C.F.R. Citation / F.R. Citation	Status of SNUR	Preamble Statement re Isocyanates	New Chemical Exposure Limit (NCEL)?	Special Restrictions in SNUR?
P-16-493	Proposed 40 C.F.R. § 721.11125 83 Fed Reg. 47004 (Sept. 17, 2018) (direct final SNUR); 83 Fed. Reg. 47026 (Sept. 17, 2018) (proposed SNUR)	Direct final SNUR withdrawn Nov. 16, 2018. Proposed SNUR under review.	§ 5(e) order: “Basis: Diisocyanates Chemicals Category NIOSH Alert on Preventing Asthma and Death from MDI Exposure During Spray-on Truck Bed Lining and Related Applications Due to the possibility of isocyanate residuals there is concern for sensitization. Exposure to diisocyanate may cause the following effects: skin irritation and allergic reaction, respiratory irritation, respiratory sensitization, and lung toxicity; some diisocyanates also may cause cancer. It is especially important to note that contact with the skin may lead to respiratory sensitization or cause other allergic reactions.”	No, but the section 5(e) order cautioned, “Inhalation exposure should be limited to < 0.05 mg/m ³ as an 8-hours time-weighted average (TWA) for combined polyisocyanates and diisocyanates.”	“It is a significant new use to import the PMN substance to contain more than 0.1% residual isocyanate by weight.”

PMN No.	C.F.R. Citation / F.R. Citation	Status of SNUR	Preamble Statement re Isocyanates	New Chemical Exposure Limit (NCEL)?	Special Restrictions in SNUR?
P-16-363	Proposed 40 C.F.R. § 721.11101 83 Fed. Reg. 43538 (Aug. 27, 2018) (direct final SNUR); 83 Fed. Reg. 63607 (Aug. 27, 2018) (proposed SNUR)	Direct final SNUR withdrawn Oct. 24, 2018. Proposed SNUR under review.	§ 5(e) order: “Toxicological Endpoints of Concern: irritation to all moist tissues and sensitization. Basis: Diisocyanate Chemicals Category NIOSH Alert on Preventing Asthma and Death from MDI Exposure During Spray-on Truck Bed Liner and Related Applications.... Also, there are concerns for lung toxicity based on cationic binding to the lung tissue.”	No	“It is a significant new use to manufacture, process, or use the substance with a residual of free isocyanate monomers greater than 0.1 percent by weight.”
P-16-99	Proposed 40 C.F.R. § 721.11098 83 Fed. Reg. 43538 (Aug. 27, 2018) (direct final SNUR); 83 Fed. Reg. 63607 (Aug. 27, 2018) (proposed SNUR)	Direct final SNUR withdrawn Oct. 24, 2018. Proposed SNUR under review.	§ 5(e) order: None specifically identified as attributable to isocyanates.	NCEL = 0.9 mg/m ³ TWA ₈	“It is a significant new use to manufacture the chemical substance containing greater than 0.2% residual isocyanate.”

PMN No.	C.F.R. Citation / F.R. Citation	Status of SNUR	Preamble Statement re Isocyanates	New Chemical Exposure Limit (NCEL)?	Special Restrictions in SNUR?
P-15-707	Proposed 40 C.F.R. § 721.11030 83 Fed. Reg. 37702 (Aug. 1, 2018) (direct final SNUR); 83 Fed. Reg. 37455 (Aug. 1, 2018) (proposed SNUR)	Direct final SNUR withdrawn Oct. 11, 2018. Proposed SNUR under review.	None in the § 5(e) order. The SNUR preamble states: “There are also concerns ... for sensitization if there are residual isocyanates.”	NCEL = 0.9 mg/m ³ TWA ₈	“A significant new use is any manufacture, processing, or use of the PMN substance with more than 1% residual isocyanate by weight.” [Note: “1%” may be an error; the preamble refers to “manufacture of the PMN substance to contain no more than 0.1% isocyanate by weight.”]
P-15-706	Proposed 40 C.F.R. § 721.11029 83 Fed. Reg. 37702 (Aug. 1, 2018) (direct final SNUR); 83 Fed. Reg. 37455 (Aug. 1, 2018) (proposed SNUR)	Direct final SNUR withdrawn Oct. 11, 2018. Proposed SNUR under review.	§ 5(e) order: None specifically identified as attributable to isocyanates. The SNUR preamble states: “There are also concerns ... for sensitization if there are residual isocyanates.”	NCEL = 0.9 mg/m ³ TWA ₈	“A significant new use is any manufacture, processing, or use of the PMN substance with more than 0.1% residual isocyanate by weight.”

PMN No.	C.F.R. Citation / F.R. Citation	Status of SNUR	Preamble Statement re Isocyanates	New Chemical Exposure Limit (NCEL)?	Special Restrictions in SNUR?
P-15-559	Proposed 40 C.F.R. § 721.10920 81 Fed. Reg. 30452 (May 16, 2016) (direct final SNUR); 81 Fed. Reg. 74755 (Oct. 27, 2016) (proposed SNUR)	Direct final SNUR withdrawn July 14, 2016. Proposed SNUR under review.	No § 5(e) order – this is a non-order SNUR. Proposed SNUR preamble: “Based on SAR analysis of analogous diisocyanates, EPA identified concerns for potential dermal and respiratory sensitization from dermal and inhalation exposures, and for pulmonary toxicity from inhalation exposure, to the PMN substance where the average molecular weight is below 7,500 daltons and any molecular weight species is below 1,000 daltons EPA has determined, however, that any manufacture of the PMN substance with an average molecular weight below 7,500 daltons, and where any molecular weight species is below 1,000 daltons may cause serious health effects. For new isocyanates submitted as PMNs, EPA expects to issue TSCA section 5(e) orders imposing 0.1% limits on total residual isocyanates and greater levels of respiratory protection (at least an APF of 50, or 1000 if used in a process that generates a vapor or particulate), and no consumer use. The Agency would then likely issue a SNUR defining the significant new use as total residual isocyanates exceeding that 0.1% limit and any use in a consumer product.”	No	“A significant new use of the substance is manufacture of the substance where the average molecular weight is below 7,500 daltons, and where any molecular weight species is below 1,000 daltons.”

PMN No.	C.F.R. Citation / F.R. Citation	Status of SNUR	Preamble Statement re Isocyanates	New Chemical Exposure Limit (NCEL)?	Special Restrictions in SNUR?
P-15-378	Proposed 40 C.F.R. § 721.10913 Direct final SNUR, 81 Fed. Reg. 30452 (May 16, 2016); proposed SNUR, 81 Fed. Reg. 74755 (Oct. 27, 2016)	Direct final SNUR withdrawn July 14, 2016. Proposed SNUR under review.	No § 5(e) order – this is a non-order SNUR. Proposed SNUR preamble: “Based on SAR analysis of test data on analogous diisocyanates, EPA identified concerns for respiratory sensitization.”	No	No
P-15-278	Proposed 40 C.F.R. § 721.10874 80 Fed. Reg. 59583 (Oct. 2, 2015) (direct final SNUR); 81 Fed. Reg. 21830 (Apr. 13, 2016) (proposed SNUR)	Direct final SNUR withdrawn Nov. 20, 2015. Proposed SNUR under review.	No § 5(e) order – this is a non-order SNUR. SNUR preamble: “Based on analogous diisocyanates, EPA identified concerns for potential dermal and respiratory sensitization from dermal and inhalation exposures, and for pulmonary toxicity from inhalation exposure, to the PMN substance when the average molecular weight is below 2500 daltons and any molecular weight species is below 1000 daltons.”	No	“The significant new use is manufacture of the substance where the average molecular weight is below 2,500 daltons and where any molecular weight species is below 1,000 daltons.”

PMN No.	C.F.R. Citation / F.R. Citation	Status of SNUR	Preamble Statement re Isocyanates	New Chemical Exposure Limit (NCEL)?	Special Restrictions in SNUR?
P-15-247	Proposed 40 C.F.R. § 721.10873 80 Fed. Reg. 59583 (Oct. 2, 2015) (direct final SNUR); 81 Fed. Reg. 21830 (Apr. 13, 2016) (proposed SNUR)	Direct final SNUR withdrawn Nov. 11, 2015. Proposed SNUR under review.	No § 5(e) order – this is a non-order SNUR. SNUR preamble: “Based on SAR analysis of test data on analogous diisocyanates, EPA identified concerns for respiratory and dermal sensitization and lung and mucous membrane irritation based on the isocyanate moiety.”	No	No
P-15-221	Proposed 40 C.F.R. § 721.10871 80 Fed. Reg. 59583 (Oct. 2, 2015) (direct final SNUR); 81 Fed. Reg. 21830 (Apr. 13, 2016) (proposed SNUR)	Direct final SNUR withdrawn Nov. 20, 2015. Proposed SNUR under review.	No § 5(e) order – this is a non-order SNUR. SNUR preamble: “Based on SAR analysis of test data on analogous diisocyanates, EPA identified concerns for irritation and sensitization to the skin and lungs.”	No	No

PMN No.	C.F.R. Citation / F.R. Citation	Status of SNUR	Preamble Statement re Isocyanates	New Chemical Exposure Limit (NCEL)?	Special Restrictions in SNUR?
P-14-357	Direct final SNUR, 40 C.F.R. § 721.10788 79 Fed. Reg. 63821 (Oct. 27, 2014)	Effective Dec. 26, 2014.	No § 5(e) order – this is a non-order SNUR. SNUR preamble: “Based on SAR analysis of test data on analogous diisocyanates, EPA identified concerns for dermal and respiratory sensitization.”	No	No
P-14-60	Proposed 40 C.F.R. § 721.10762 79 Fed. Reg. 38464 (July 8, 2014) (direct final SNUR); 80 Fed. Reg. 858 (Jan. 9, 2015) (proposed SNUR)	Direct final SNUR withdrawn Sept. 4, 2014. Proposed SNUR under review.	No § 5(e) order – this is a non-order SNUR. SNUR preamble: “Based on SAR analysis of test data on analogous diisocyanates, EPA identified concerns for dermal and respiratory sensitization to persons exposed to the PMN substance.”	No	No

PMN No.	C.F.R. Citation / F.R. Citation	Status of SNUR	Preamble Statement re Isocyanates	New Chemical Exposure Limit (NCEL)?	Special Restrictions in SNUR?
P-13-619	Proposed 40 C.F.R. § 721.10744 79 Fed. Reg. 39268 (July 9, 2014) (direct final rule); 80 Fed. Reg. 858 (Jan. 7, 2015) (proposed SNUR)	Direct final SNUR withdrawn Sept. 4, 2014. Proposed SNUR under review.	No § 5(e) order – this is a non-order SNUR. SNUR preamble: “Based on SAR analysis of test data on analogous diisocyanates, EPA identified concerns for respiratory sensitization.”	No	No
P-13-618	Proposed 40 C.F.R. § 721.10743 79 Fed. Reg. 39268 (July 9, 2014) (direct final rule); 80 Fed. Reg. 858 (Jan. 7, 2015) (proposed SNUR)	Direct final SNUR withdrawn Sept. 4, 2014. Proposed SNUR under review.	No § 5(e) order – this is a non-order SNUR. SNUR preamble: “Based on SAR analysis of test data on analogous diisocyanates, EPA identified concerns for respiratory sensitization.”	No	No

PMN No.	C.F.R. Citation / F.R. Citation	Status of SNUR	Preamble Statement re Isocyanates	New Chemical Exposure Limit (NCEL)?	Special Restrictions in SNUR?
P-13-617	Proposed 40 C.F.R. § 721.10742 79 Fed. Reg. 39268 (July 9, 2014) (direct final rule); 80 Fed. Reg. 858 (Jan. 7, 2015) (proposed SNUR)	Direct final SNUR withdrawn Sept. 4, 2014. Proposed SNUR under review.	No § 5(e) order – this is a non-order SNUR. SNUR preamble: “Based on SAR analysis of test data on analogous diisocyanates, EPA identified concerns for respiratory sensitization.”	No	No
P-13-563	Proposed 40 C.F.R. § 721.10741 79 Fed. Reg. 39268 (July 9, 2014) (direct final rule); 80 Fed. Reg. 858 (Jan. 7, 2015) (proposed SNUR)	Direct final SNUR withdrawn Sept. 4, 2014. Proposed SNUR under review.	No § 5(e) order – this is a non-order SNUR. SNUR preamble: “Based on SAR analysis of analogous diisocyanates, EPA identified concerns for oncogenicity, mutagenicity, respiratory and dermal sensitization and lung and mucous membrane irritation from exposure to the PMN substance via inhalation and dermal exposures.”	No	No

PMN No.	C.F.R. Citation / F.R. Citation	Status of SNUR	Preamble Statement re Isocyanates	New Chemical Exposure Limit (NCEL)?	Special Restrictions in SNUR?
P-13-559	40 C.F.R. § 721.10795 80 Fed. Reg. 5457 (Feb. 2, 2015) (direct final SNUR)	Effective Apr. 3, 2015.	No § 5(e) order – this is a non-order SNUR. None specifically identified in SNUR preamble as attributable to isocyanates.	No	No
P-13-471	Proposed 40 C.F.R. § 721.10723 79 Fed. Reg. 8273 (Feb. 12, 2014) (direct final SNUR); 80 Fed. Reg. 845 (Jan. 7, 2015) (proposed SNUR)	Direct final SNUR withdrawn Apr. 14, 2014. Proposed SNUR under review.	No § 5(e) order – this is a non-order SNUR. SNUR preamble: “Based on test data on analogous diisocyanates, EPA identified concerns for oncogenicity, mutagenicity, respiratory and dermal sensitization, and lung and mucous membrane irritation to workers exposed to the PMN substance.”	No	No

PMN No.	C.F.R. Citation / F.R. Citation	Status of SNUR	Preamble Statement re Isocyanates	New Chemical Exposure Limit (NCEL)?	Special Restrictions in SNUR?
P-13-393	Proposed 40 C.F.R. § 721.10720 79 Fed. Reg. 8273 (Feb. 12, 2014) (direct final SNUR); 80 Fed. Reg. 845 (Jan. 7, 2015) (proposed SNUR)	Direct final SNUR withdrawn Apr. 14, 2014. Proposed SNUR under review.	No § 5(e) order – this is a non-order SNUR. SNUR preamble: “Based on test data on analogous diisocyanates, the Agency identified concerns for dermal and respiratory sensitization, irritation to all moist tissues, and lung effects if inhaled based on the low molecular weight isocyanates.”	No	No
P-13-392	Proposed 40 C.F.R. § 721.10719 79 Fed. Reg. 8273 (Feb. 12, 2014) (direct final SNUR); 80 Fed. Reg. 845 (Jan. 7, 2015) (proposed SNUR)	Direct final SNUR withdrawn Jan. 14, 2014. Proposed SNUR under review.	No § 5(e) order – this is a non-order SNUR. SNUR preamble: “EPA identified concerns for dermal and respiratory sensitization, irritation to all moist tissues, and lung effects if inhaled based on the low molecular weight isocyanates, to workers exposed to the PMN substance.”	No	No

PMN No.	C.F.R. Citation / F.R. Citation	Status of SNUR	Preamble Statement re Isocyanates	New Chemical Exposure Limit (NCEL)?	Special Restrictions in SNUR?
P-13-365	Proposed 40 C.F.R. § 721.10717 79 Fed. Reg. 8273 (Feb. 12, 2014) (direct final SNUR); 80 Fed. Reg. 845 (Jan. 7, 2015) (proposed SNUR)	Direct final SNUR withdrawn Apr. 14, 2014. Proposed SNUR under review.	No § 5(e) order – this is a non-order SNUR. SNUR preamble: “Based on test data on analogous diisocyanates, EPA identified concerns for dermal and respiratory sensitization, and lung and mucous membrane irritation effects.”	No	No
P-13-357	40 CF.R. § 721.10788 78 Fed. Reg. 63821 (Oct. 27, 2014)	Effective Dec. 26, 2014	No § 5(e) order – this is a non-order SNUR. SNUR preamble: “Based on SAR analysis of test data on analogous diisocyanates, EPA identified concerns for irritation to the eye, skin, and mucous membranes; and dermal and respiratory sensitization.”	No	No
P-13-338	40 CF.R. § 721.10693 78 Fed. Reg. 48059 (Aug. 7, 2013)	Effective Oct. 7, 2013.	No § 5(e) order – this is a non-order SNUR. SNUR preamble: “Based on analogous diisocyanates, EPA identified concerns for dermal and respiratory sensitization, irritation, and lung effects.”	No	No

PMN No.	C.F.R. Citation / F.R. Citation	Status of SNUR	Preamble Statement re Isocyanates	New Chemical Exposure Limit (NCEL)?	Special Restrictions in SNUR?
P-13-232	40 C.F.R. § 721.10690 78 Fed. Reg. 48059 (Aug. 7, 2013) (direct final SNUR)	Effective Oct. 7, 2013.	No § 5(e) order – this is a non-order SNUR. SNUR preamble: “Based on analogous diisocyanates, EPA identified concerns for sensitization as well as lung and mucous membrane irritation.”	No	No
P-13-176	40 C.F.R. § 721.10773 79 Fed. Reg. 63821 (Oct. 27 2014) (direct final SNUR)	Effective Dec. 26, 2014	No § 5(e) order – this is a non-order SNUR. None specifically identified in SNUR preamble as attributable to isocyanates.	No	No
P-13-175	40 C.F.R. § 721.10772 79 Fed. Reg. 63821 (Oct. 27, 2014) (direct final SNUR)	Effective Dec. 26, 2014	No § 5(e) order – this is a non-order SNUR. None specifically identified in SNUR preamble as attributable to isocyanates.	No	No
P-12-373	40 C.F.R. § 721.10626 77 Fed. Reg. 66149 (Nov. 2, 2012) (direct final SNUR)	Effective Jan. 2, 2013	No § 5(e) order – this is a non-order SNUR. SNUR preamble: “Based on test data on analogous acrylates and isocyanates, EPA identified concerns for respiratory and dermal sensitization and irritation to workers from exposure to the PMN substance.”	No	No

PMN No.	C.F.R. Citation / F.R. Citation	Status of SNUR	Preamble Statement re Isocyanates	New Chemical Exposure Limit (NCEL)?	Special Restrictions in SNUR?
P-12-326	40 C.F.R. § 721.10624 77 Fed. Reg. 66149 (Nov. 2, 2012) (direct final SNUR)	Effective Jan. 2, 2013	No § 5(e) order – this is a non-order SNUR. SNUR preamble: “Based on analogous diisocyanate substances, EPA identified concerns for potential dermal and respiratory sensitization from dermal and inhalation exposures, and for pulmonary toxicity from inhalation exposure to the PMN substance. Specifically, the Agency expects potential toxicity to workers from dermal or inhalation exposure to the PMN substance when the molecular weight is less than 1000 daltons.”	No	“The significant new uses are: (i) Industrial, commercial, and consumer activities. Requirements as specified in § 721.80(j) (manufacture, processing, or use where the molecular weight is 1000 daltons or more).” [Note: presumably should be 1000 daltons or less.]
P-12-274	40 C.F.R. § 721.10660 79 Fed. Reg. 51899 (Sept. 2, 2014) (final SNUR); 78 Fed. Reg. 12684 (Feb. 25, 2013) (proposed SNUR)	Effective Nov. 3, 2014	No § 5(e) order – this is a non-order SNUR. Proposed SNUR preamble: “Based on SAR analysis of test data on analogous isocyanates, EPA identified concern for sensitization from dermal and inhalation exposure to the PMN substance.”	No	No

PMN No.	C.F.R. Citation / F.R. Citation	Status of SNUR	Preamble Statement re Isocyanates	New Chemical Exposure Limit (NCEL)?	Special Restrictions in SNUR?
P-12-143	40 C.F.R. § 721.10659 79 Fed. Reg. 51899 (Sept. 2, 2014) (final SNUR); 78 Fed. Reg. 12684 (Feb. 25, 2013) (proposed SNUR)	Effective Nov. 3, 2014	No § 5(e) order – this is a non-order SNUR. Proposed SNUR preamble: “Based on SAR analysis of test data on analogous isocyanates, EPA identified concerns for sensitization from dermal and respiratory exposures to the PMN substance.”	No	No
P-12-133	40 C.F.R. § 721.10658 79 Fed. Reg. 51899 (Sept. 2, 2014) (final SNUR); 78 Fed. Reg. 12684 (Feb. 25, 2013) (proposed SNUR)	Effective Nov. 3, 2014	No § 5(e) order – this is a non-order SNUR. Proposed SNUR preamble: “based on the isocyanate moiety, the Agency identified concerns for sensitization.”	No	No

PMN No.	C.F.R. Citation / F.R. Citation	Status of SNUR	Preamble Statement re Isocyanates	New Chemical Exposure Limit (NCEL)?	Special Restrictions in SNUR?
P-12-73	40 C.F.R. § 721.10657 79 Fed. Reg. 51899 (Sept. 2, 2014) (final SNUR); 78 Fed. Reg. 12684 (Feb. 25, 2013) (proposed SNUR)	Effective Nov. 3, 2014	No § 5(e) order – this is a non-order SNUR. Proposed SNUR preamble: “Based on SAR analysis of test data on analogous diisocyanates, EPA identified concerns for sensitization.”	No	No
P-11-862	40 C.F.R. § 721.10298 77 Fed. Reg. 20296 (Apr. 4, 2012) (direct final SNUR)	Effective June 4, 2012	No § 5(e) order – this is a non-order SNUR. SNUR preamble: “Based on test data on analogous diisocyanates, EPA identified concerns for mutagenicity, irritation to lungs and mucous membranes, and dermal and respiratory sensitization to workers from dermal and inhalation exposure to the PMN substance.”	No	No

PMN No.	C.F.R. Citation / F.R. Citation	Status of SNUR	Preamble Statement re Isocyanates	New Chemical Exposure Limit (NCEL)?	Special Restrictions in SNUR?
P-11-314	40 C.F.R. § 721.10656 79 Fed. Reg. 51899 (Sept. 2, 2014) (final SNUR); 78 Fed. Reg. 12684 (Feb. 25, 2013) (proposed SNUR)	Effective Nov. 3, 2014	No § 5(e) order – this is a non-order SNUR. Proposed SNUR preamble: “Based on SAR analysis of test data on analogous isocyanates, EPA identified concerns for sensitization from dermal and inhalation exposure to the PMN substances.”	No	No
P-11-313	40 C.F.R. § 721.10655 79 Fed. Reg. 51899 (Sept. 2, 2014) (final SNUR); 78 Fed. Reg. 12684 (Feb. 25, 2013) (proposed SNUR)	Effective Nov. 3, 2014	No § 5(e) order – this is a non-order SNUR. Proposed SNUR preamble: “Based on SAR analysis of test data on analogous isocyanates, EPA identified concerns for sensitization from dermal and inhalation exposure to the PMN substances.”	No	No

PMN No.	C.F.R. Citation / F.R. Citation	Status of SNUR	Preamble Statement re Isocyanates	New Chemical Exposure Limit (NCEL)?	Special Restrictions in SNUR?
P-11-312	40 C.F.R. § 721.10654 79 Fed. Reg. 51899 (Sept. 2, 2014) (final SNUR); 78 Fed. Reg. 12684 (Feb. 25, 2013) (proposed SNUR)	Effective Nov. 3, 2014	No § 5(e) order – this is a non-order SNUR. Proposed SNUR preamble: “Based on SAR analysis of test data on analogous isocyanates, EPA identified concerns for sensitization from dermal and inhalation exposure to the PMN substances.”	No	No
P-11-311	40 C.F.R. § 721.10653 79 Fed. Reg. 51899 (Sept. 2, 2014) (final SNUR); 78 Fed. Reg. 12684 (Feb. 25, 2013) (proposed SNUR)	Effective Nov. 3, 2014	No § 5(e) order – this is a non-order SNUR. Proposed SNUR preamble: “Based on SAR analysis of test data on analogous isocyanates, EPA identified concerns for sensitization from dermal and inhalation exposure to the PMN substances.”	No	No

PMN No.	C.F.R. Citation / F.R. Citation	Status of SNUR	Preamble Statement re Isocyanates	New Chemical Exposure Limit (NCEL)?	Special Restrictions in SNUR?
P-11-309	40 C.F.R. § 721.10652 79 Fed. Reg. 51899 (Sept. 2, 2014) (final SNUR); 78 Fed. Reg. 12684 (Feb. 25, 2013) (proposed SNUR)	Effective Nov. 3, 2014	No § 5(e) order – this is a non-order SNUR. Proposed SNUR preamble: “Based on SAR analysis of test data on analogous isocyanates, EPA identified concerns for sensitization from dermal and inhalation exposure to the PMN substances.”	No	No
P-11-115	40 C.F.R. § 721.10649 79 Fed. Reg. 51899 (Sept. 2, 2014) (final SNUR); 78 Fed. Reg. 12684 (Feb. 25, 2013) (proposed SNUR)	Effective Nov. 3, 2014	No § 5(e) order – this is a non-order SNUR. Proposed SNUR preamble: “Based on SAR analysis of test data on analogous diisocyanates, EPA identified concerns for respiratory and dermal sensitization.”	No	No

PMN No.	C.F.R. Citation / F.R. Citation	Status of SNUR	Preamble Statement re Isocyanates	New Chemical Exposure Limit (NCEL)?	Special Restrictions in SNUR?
P-11-60	40 C.F.R. § 721.10661 78 Fed. Reg. 27048 (May 9, 2013) (direct final SNUR)	Effective July. 8, 2013.	No § 5(e) order – this is a non-order SNUR. SNUR preamble: “Based on structural activity relationship (SAR) analysis of test data on analogous diisocyanates, EPA identified concerns for dermal and respiratory sensitization and for pulmonary toxicity to workers exposed to free isocyanates.”	No	No
P-08-611	40 C.F.R. § 721.10571 77 Fed. Reg. 61118 (Oct. 5, 2012) (direct final SNUR)	Effective Dec. 4, 2012.	No § 5(e) order – this is a non-order SNUR. SNUR preamble: “Based on SAR analysis of test data on analogous diisocyanates, EPA identified concerns for dermal and respiratory sensitization and for pulmonary toxicity to workers exposed to free isocyanates.”	No	Significant new use is manufacture other than as described in the PMN, “(manufacture with all isocyanate groups reacted within the polymer).”
P-04-834	40 C.F.R. § 721.10490 77 Fed. Reg. 58666 (Sept. 21, 2012) (direct final rule)	Effective Nov. 20, 2012	§ 5(e) order unavailable. Direct final SNUR preamble: “Based on test data on analogous diisocyanates, EPA identified concerns for dermal and respiratory sensitization, pulmonary toxicity, and carcinogenicity from dermal and inhalation exposures.”	NCEL = 0.05 mg/m ³ TWA ₈	No

PMN No.	C.F.R. Citation / F.R. Citation	Status of SNUR	Preamble Statement re Isocyanates	New Chemical Exposure Limit (NCEL)?	Special Restrictions in SNUR?
P-04-640	40 C.F.R. § 721.10643 79 Fed. Reg. 51899 (Sept. 2, 2014) (final SNUR); 78 Fed. Reg. 12684 (Feb. 25, 2013) (proposed SNUR)	Effective Nov. 3, 2014	§ 5(e) order unavailable. Proposed SNUR preamble: “Based on SAR analysis of test data on structurally similar diisocyanates, EPA identified concerns for dermal sensitization, respiratory sensitization, and pulmonary toxicity from exposure to the PMN substance by the inhalation and dermal routes.”	No	No
P-04-563	40 C.F.R. § 721.10343 77 Fed. Reg. 25236 (Apr. 27, 2012)	Effective June 26, 2012	No § 5(e) order – this is a non-order SNUR. SNUR preamble: None specifically identified as attributable to isocyanates.	No	No
P-03-767	40 C.F.R. § 721.10331	Effective June 26, 2012	No § 5(e) order – this is a non-order SNUR. SNUR preamble: None specifically identified as attributable to isocyanates.	No	No

PMN No.	C.F.R. Citation / F.R. Citation	Status of SNUR	Preamble Statement re Isocyanates	New Chemical Exposure Limit (NCEL)?	Special Restrictions in SNUR?
P-03-762 and P-03-763	40 C.F.R. § 721.10642 79 Fed. Reg. 51899 (Sept. 2, 2014) (final SNUR); 78 Fed. Reg. 12684 (Feb. 25, 2013) (proposed SNUR)	Effective Nov. 3, 2014	No § 5(e) order – this is a non-order SNUR. Proposed SNUR preamble: “Based on SAR analysis of test data on analogous isocyanates, EPA has identified concerns for sensitization and irritation from dermal and inhalation exposure to the PMN substances.”	No	No

Attachment 2

Isocyanates Are Not a Leading Cause of Occupational Asthma

April 5, 2019

Isocyanates Are Not a Leading Cause of Occupational Asthma

EPA has asserted multiple times that:

Isocyanate exposure has been identified as the leading attributable cause of work-related asthma, and prevalence in the exposed workforce has been estimated at 1-20%

This statement was not accurate when originally made, and it has become increasingly inaccurate with time. This paper first summarizes EPA's extensive reliance on the statement quoted above. Next, it reviews the sources on which the quoted statement relies and shows that those sources do not support the statement. It ends with a review of recent evidence that isocyanates are not now a significant cause of occupational asthma and have a low incidence rate.

In light of this evidence, EPA should stop referring to isocyanates as the leading attributable cause of occupational asthma. It should also stop referring to outdated and misleading incidence rates of isocyanate-related asthma.

1. EPA Has Repeatedly Referred to Isocyanates as the Leading Cause of Occupational Asthma, With Incidence of Up to 20%

In April 2011, EPA posted a Methylene Diphenyl Diisocyanate (MDI) And Related Compounds Action Plan and a Toluene Diisocyanate (TDI) And Related Compounds Action Plan. Both action plans asserted (p. 4):

It is well documented that isocyanate exposure is the leading cause of work-related asthma, and prevalence in the exposed workforce is estimated at 1-20% (Ott et al., 2003;¹ Bello et al., 2004).²

EPA repeated that statement in its proposed SNUR for TDI, 80 Fed. Reg. 2068, 2070 (Jan. 15, 2015):

Isocyanate exposure has been identified as the leading attributable cause of work-related asthma, and prevalence in the exposed workforce has been estimated at 1–20 percent (Refs. 11 and 12).³

¹ The citation for the Ott et al. paper is Ott MG, Diller, WF, Jolly AT. 2003. Respiratory Effects of Toluene Diisocyanates in the Workplace; a Discussion of Exposure-Response Relationships. *Critical Review Toxicology* 33:1-59.

² The citation for the Bello et al. paper is Bello D, Woskie SR, Streicher RP, Liu Y, Stowe MH, Eisen EA, Ellenbecker MJ, Sparer J, Youngs F, Cullen MR, Redlich CA. 2004. Polyisocyanates in Occupational Environments: a Critical Review of Exposure Limits and Metrics. *American Journal Industrial Medicine* 46:480-491.

³ Reference 11 and 12 are to the Ott et al. and Bello et al. papers cited above.

The statement appeared again in EPA's 2017 section 5(f) order for two new isocyanate-based polymers, P-17-24 and P-17-25. It stated (p. vii):

Isocyanate exposure has been identified as the leading attributable cause of work-related asthma, and prevalence in the exposed workforce has been estimated at 1-20 percent (see Refs. 1 and 2).⁴

In addition, EPA has issued several section 5(e) orders⁵ for other new isocyanate-based polymers that refer to the 2006 NIOSH Alert, "Preventing Asthma and Death from MDI Exposure During Spray-on Truck Bed Liner and Related Applications," which states (p. 4):

Isocyanates are the leading attributable chemical cause of occupational asthma in the United States and many other industrialized countries [Tarlo et al. 1997b].

With these statements reasserted so many times, it is likely that EPA relies on them for the restrictions it imposes on new isocyanate-based polymers it reviews under section 5 of TSCA. As demonstrated in section 2 below, the statements were not accurate at the time they were originally made. As demonstrated in section 3 below, the statements are even less accurate now.

2. The Cited References Do Not Support the EPA Statements

The sources on which EPA relied for the statements noted in section 1 are more than 10 years old. They relied in turn on older papers, which cited even older papers. The Ott et al., Bello et al., and Tarlo et al. papers do not support the statements repeated by EPA.

a. Ott et al. (2003)

The 2003 Ott et al. paper, now 16 years old, was a literature review, not a primary source. It presented a very different perspective than that cited by EPA:

In the early years of the industry, annual incidence rates of occupational asthma (OA) due to TDI ranged from 1% to as high as 6%, depending on the extent of engineering and work practice controls in the various workplaces. **Since the mid-1970s, annual OA incidence rates have been < 1%**, where 8 h TDI concentrations have been maintained below 5 ppb as determined by personal monitoring, even where short-term TDI concentrations above 20 ppb and less frequently above 40 ppb were routinely detected. [Emphasis added.]

The paper did say that "diisocyanates are among the most prevalent reported causes of OA." It did not mention a 20% incidence rate, however.

b. Bello et al. (2004)

⁴ References 1 and 2 are to the Ott et al. and Bello et al. papers cited above.

⁵ Including the section 5(e) orders for PMNs P-17-231, P-17-222, P-17-24 and -25, 16-493, and 16-393.

This paper did use a 20% incidence figure for sensitization and asthma together (i.e., not specifically for asthma), citing other older studies of which none suggested an incidence rate of 20%:

However, sensitization and asthma are the primary health concerns, and **their estimated prevalence in the exposed workforce is 1-20%** [Vandenplas et al., 1993a; Bernstein, 1996; Petsonk et al., 2000; Wisnewski and Redlich, 2001; Diller, 2002]. [Emphasis added.]

The cited papers do not support the Bello et al. statement of an incidence rate of up to 20%.

The 1993 Vandenplas et al. paper,⁶ itself a literature review and now 26 years old, did not support a 20% incidence figure. It stated:

According to the results of studies with objective diagnostic tests, a **prevalence of about 10%** seems to be a reasonable approximation. [Citations omitted, emphasis added.]

It did state that “isocyanates are the principal cause of occupational asthma,” citing a 1984 paper by Davies (now 35 years old). The Davies paper did not summarize surveillance data; it included only the author’s opinion.

The 1996 Bernstein et al. paper⁷ was also a literature review and is now 23 years old. It did not suggest a ranking of the leading causes of occupational asthma. The paper suggested a prevalence of 5 to 10%:

Surveillance programs established around the world have determined that diisocyanate chemicals are the most common cause of occupational asthma. In the United States approximately 100,000 workers are exposed to these chemical compounds in the workplace each year and **5-10% of these workers will develop occupational asthma**. [Emphasis added.]

The 2000 Petsonk et al. paper,⁸ now 19 years old, did not indicate the ranking of diisocyanates with other causes of occupational asthma. It indicated a “low prevalence” where exposures are controlled:

Diisocyanates, a group of highly reactive chemicals, have been frequently associated with the new onset of asthma in relation to work exposures, although in industrial settings in which exposures to these chemicals are well controlled, **a low prevalence of symptoms has been reported**. [Emphasis added.]

⁶ The citation is Vandenplas O, Malo JL, Saetta M, Mapp CE, Fabbri LM. 1993. Occupational asthma and extrinsic alveolitis due to isocyanates; current status and perspectives. *Br J Ind Med* 50:213-228.

⁷ The citation is Bernstein, JA. 1996. Overview of diisocyanate occupational asthma. *Toxicology* 111(1-3):181-189.

⁸ The citation is Petsonk EL, Wang ML, Lewis DM, Siegel PD, Husberg BJ. 2000. Asthma-like symptoms in wood product plant workers exposed to methylene diphenyl diisocyanate. *Chest* 118:1183-1193.

The 2001 Wisnewski and Redlich paper,⁹ now 18 years old, referenced 11 papers in stating, “Diisocyanates are one of the most commonly identified causes of occupational asthma.” However, only one of the references, a 1999 paper by Jajosky et al.¹⁰ (now 20 years old), actually ranked the causes. Using data from 1993-1995 (24 to 26 years ago), it listed TDI as number 8 (3.7% of cases); diisocyanates NOS as number 10 (3.3% of cases); and MDI as number 15 (2.4% of cases).

The Wisnewski and Redlich paper estimated prevalence of isocyanate-caused occupational asthma at between 5 and 15%, citing earlier papers.

The 2002 Diller paper¹¹ (now 17 years old) did not refer to isocyanates as the leading cause of occupational asthma. Instead, in referencing a 1993 book (now 26 years old), it said:

In recent years, occupational asthma (OA) has become the most frequent occupational lung disease in many countries. Among the many agents that may cause OA, the isocyanates have gained wide attention.

The Diller paper indicated that incidence of TDI-caused occupational asthma was between 0 and 10% as of 2002:

Prevalence of occupational asthma due to toluene diisocyanate can be estimated from 10 cross-sectional studies, based on 788 persons. Prevalence had repeatedly been above 10 percent before 1985, and have been mostly between zero and 10 percent in recent years.

It added:

According to general surveillance schemes, compensation statistics, and disease registers, the annual case numbers of OA due to all types of isocyanates also show a downward trend in most countries during recent years, in spite of steadily increasing production and use of all isocyanates.

In short, the sources cited in the 2003 Ott et al. paper and the 2004 Bello et al. paper do not support the assertions that EPA has repeatedly made.

⁹ The citation is Wisnewski AV, Redlich CA. 2001. Recent developments in diisocyanate asthma. *Curr Opin Allergy Clin Immunol* 1:169-175.

¹⁰ The citation is Jajosky RA, Harrison R, Reinisch F, et al. Surveillance of work-related asthma in selected US states using surveillance guidelines for state health departments ± California, Massachusetts, Michigan, and New Jersey, 1993-1995. *Morb Mortal Weekly Rep CDC Surveillance Summaries* 1999; 48:1-20.

¹¹ The citation is Diller WF. 2002. Frequency and trends of occupational asthma due to toluene diisocyanate: A critical review. *Appl Occup Environ Hyg* 17:872-877.

c. **Tarlo et al. (1997)**

The 2006 NIOSH Alert cited only Tarlo et al.¹² for its assertion that “isocyanates are the leading attributable chemical cause of occupational asthma in the United States and many other industrialized countries.” This 1997 article appeared 22 years ago. While ACC has not reviewed the full article, its abstract reports that the paper “provides some evidence that facilities having OA claims have higher isocyanate exposures than to those without claims.” This suggests that any statement about isocyanates being the leading attributable chemical cause of occupational asthma was supported only by references to even earlier papers, as was the case with the Ott et al. paper and the Bello et al. paper. These papers relied on evidence now more than two decades old. They do not reflect current incidence rates, as demonstrated below.

Notably, much more recently, Tarlo has co-authored two articles that reported substantial declines in the isocyanate-related occupational asthma cases. The 2011 article¹³ found:

In conclusion, the study suggests that there has been a reduction in absolute number of ISO and N-ISO OA allowed claims, with a somewhat greater relative decline of ISO OA claims.

The 2016 article¹⁴ stated:

The recent period included a significantly smaller proportion (of OA cases) employed in the manufacturing industry and isocyanate-induced cases compared with the earlier period.

It established that there have only been 3 cases of diisocyanate-related occupational asthma in the years 2010-2014 in Toronto, Canada.

Thus, EPA should not continue to rely on a 2006 NIOSH alert that cites a 22-year-old paper whose lead author has since written at least twice on the substantial decline in the incidence of isocyanate-related occupational asthma.

3. Current Information Shows That Isocyanates Are Not a Significant Cause of Occupational Asthma

The outdated references on which EPA relies for its misleading statements about isocyanates and occupational asthma do not reflect the sharp drop in incidence of isocyanate-related occupational asthma. Current information establishes that isocyanates are now a minor cause of occupational asthma.

¹² The citation is Tarlo SM, Liss GM, Dias C, Banks DE. 1997. Assessment of the relationship between isocyanate exposure levels and occupational asthma. *Am J Ind Mem* 32(5):517-5 21.

¹³ The citation is Buyantseva L, Liss GM, Ribeiro, Manno M, Luce CE, Tarlo SM. 2011. Reduction in Diisocyanate and Non-Diisocyanate Sensitizer-Induced Occupational Asthma in Ontario. *JOEM*. DOI: 10.1097/JOM.0b013e3182122376.

¹⁴ The citation is Gotzev G, Lipszyc JC, Connor D, Tarlo SM. 2016. Trends in Occupations and Work Sectors Among Patients With Work-Related Asthma at a Canadian Tertiary Care Clinic. *Chest*. 150(4):811-818.

The NIOSH website reports on most-frequently reported causes of occupational asthma for the period of 2009-2012 in four states. The leading cause was reported to be “miscellaneous chemicals and materials” (not including isocyanates), accounting for 22.4% of the cases. Isocyanates were the 19th most-frequently reported cause, accounting for just 1.0% of the cases. This information contradicts the assertion in the 2006 NIOSH Alert.

A 2017 paper by Collins et al.¹⁵ reported that “diisocyanates, such as toluene diisocyanate (TDI), are a cause of occupational asthma.” It then explains that incidence rates for TDI-induced asthma have declined:

There is evidence from surveillance reports of declining trends in occupational asthma during the 1990s in the United States, United Kingdom, Finland, and Canada. Reviews of workplace studies indicate also that incidence rates of TDI-induced asthma have declined. These favorable trends appear to be related to a reduction in workplace exposures through engineering controls and changes in work practices.

A 2015 paper by Stocks¹⁶ et al. made its own finding about declining incidence rates:

From 2006 to 2014, there was a significant decline in the number of urine samples with detectable levels of [a biomarker for exposure to 1,6-hexamethylene diisocyanate (HDI)] Over the same period, there was a significant decline in all asthma cases attributed to isocyanates or spray painting reported to SWORD ... and a non-significant decline among MVR workers

This paper attributed the decline to improved industrial hygiene methods:

The simultaneous decrease in HDI exposure and incident cases of asthma reported to SWORD is temporally consistent with a reduction in exposure to airborne isocyanate leading to a reduction in asthma. Although this is not direct evidence of a causal relationship between the two trends, it is suggestive.

Declines were noted even in papers from a decade earlier. As noted above, the 2003 Ott et al. paper reported:

In the early years of the industry, annual incidence rates of occupational asthma (OA) due to TDI ranged from 1% to as high as 6%, depending on the extent of engineering and work practice controls in the various workplaces. Since the mid-1970s, annual OA incidence rates have been < 1%, where 8 h TDI concentrations have been maintained below 5 ppb as determined by personal monitoring, even where short-term TDI concentrations above 20 ppb and less frequently above 40 ppb were routinely detected.

¹⁵ The citation is Collins JJ, Anteau S, Conner PR, Cassidy LD, Doney B, Wang ML, Kurth L, Carson M, Molenaar D, Redlich CA, Storey E. 2017. Incidence of Occupational Asthma and Exposure to Toluene Diisocyanate in the United States Toluene Diisocyanate Production Industry. JOEM 59(125):S22-S27.

¹⁶ The citation is Stocks SJ, Jones K, Piney M, Agius RM. 2015. Isocyanate exposure and asthma in the UK vehicle repair industry. Occupational Medicine 2015;65:713-718.

In addition to improved industrial hygiene practices, substitution of MDI for TDI may also have contributed to the decline in incidence of isocyanate-related asthma. A 2005 paper by Krone et al.¹⁷ noted:

Prior to 1980, most PUF [polyurethane foam] was produced using toluene diisocyanate (TDI). Post-1980, there was a shift to the use of methylenediphenyl diisocyanate (MDI) and pre-polymers of TDI (pre-reacted TDI polymer) MDI was introduced as a safer substitute for TDI in the manufacture of PU due to MDI's exceedingly low volatility¹⁸ and correspondingly low inhalation potential (less than 1 ppb on average).

Recent data compiled by ACC show a consistent picture of a decline in asthma rates associated with diisocyanates over the last decade, even as production rates of diisocyanates have increased. The reduction in diisocyanate-related occupational asthma is primarily due to a variety of product stewardship activities, including education and training, enhanced worker awareness, improved work practices, use of less volatile diisocyanate forms (e.g., pre-polymers), improved engineering controls (e.g., containment and/or ventilation), better medical surveillance programs, minimization of peak exposures, and continuing emphasis on compliance with existing exposure standards. These product stewardship efforts are key to further reductions in cases.

Conclusion

EPA's repeated statements about isocyanates being the leading cause of occupational asthma and incidence rates of isocyanate-related asthma as high as 20% are inaccurate and based on assessments primarily from the 1990s and earlier. They do not reflect changes in industrial hygiene practices and switching from TDI to MDI in many applications. Current evidence indicates that isocyanates are not a leading cause of occupational asthma. They account for 1% or less of occupational asthma cases.

Accordingly, EPA should stop repeating those statements. Instead, it should review current scientific literature. It should make its regulatory decisions under section 5 in light of current information, not on the basis of outdated and inaccurate papers.

¹⁷ The citation is Krone CA, Klinger TD. 2005. Isocyanates, polyurethane and childhood asthma. *Pediatric Allergy Immunol* 2005;16:368-379.

¹⁸ TDI has a vapor pressure of 0.01 mm Hg, whereas MDI has a vapor pressure of 0.000005 mm Hg, four orders of magnitude lower. See the NIOSH Pocket Guide to Chemical Hazards.

AMERICAN CHEMISTRY COUNCIL ENVIRONMENTAL PROTECTION AGENCY MEETING AGENDA

Date: Wednesday, April 10, 2019
Time: 8:00 – 9:00 am Eastern
Location: EPA East Building
1200 Constitution Avenue, NW
Washington, DC 20004

Time	Topic	Lead
8:00 a.m.	Administrative <ul style="list-style-type: none">• Introductions• Background• Purpose of Meeting	S. Osman-Sypher, ACC C. Franz, ACC
8:10 a.m.	Discuss ACC Questions on EPA's Approach to Isocyanate-Based Polymers Under Section 5 of TSCA	S. Osman-Sypher, ACC C. Franz, ACC
8:50 a.m.	Discuss Next Steps	S. Osman-Sypher, ACC C. Franz, ACC
9:00 a.m.	Adjournment	

ANTITRUST CHECKLIST FOR AMERICAN CHEMISTRY COUNCIL MEETINGS

This antitrust checklist, a part of ACC's Antitrust Compliance Guide, is for use by ACC staff and member company representatives in the conduct of ACC-sponsored meetings. Prohibited discussion topics apply equally to social gatherings incidental to ACC-sponsored meetings. The checklist is not exhaustive and does not address antitrust issues relating to activities other than ACC meetings. Participants in ACC meetings also should be thoroughly familiar with the Antitrust Compliance Guide.

DO

Do ensure strict performance in areas of:

OVERSIGHT/SUPERVISION:

- Have an ACC staff representative at each ACC-sponsored meeting;
- Consult with ACC counsel on all antitrust questions relating to ACC-sponsored meetings;
- Limit meeting discussions to agenda topics (unless additional topics have been approved by the ACC staff representative); and
- Provide each member company representative and ACC employee attending an ACC-sponsored meeting with a copy of this checklist, and have a copy available for reference at all ACC-sponsored meetings.

RECORDKEEPING:

- Have an agenda and minutes which accurately reflect the matters which occur; and
- Provide agendas and minutes to ACC legal counsel for review and approval in advance of distribution.

VIGILANCE:

- Protest against or stop any discussion or meeting activities which appear to violate this checklist. Member company representatives should disassociate themselves from any such discussion or activities and leave any meeting in which they continue.

DON'T

Don't, in fact or appearance, discuss or exchange information on:

PRICES, INCLUDING:

- Individual company prices, price changes, price differentials, markups, discounts, allowance, credit terms, etc.;
- Individual company data on costs, production, capacity, inventories, sales, etc.; and
- Industry pricing policies, price levels, price changes, differentials, etc.

PRODUCTION, INCLUDING:

- Plans of individual companies concerning the design, production, distribution or marketing of particular products, including proposed territories or customers; and
- Changes in industry production, capacity or inventories.

TRANSPORTATION RATES:

- Rates or rate policies for individual shipments, including basing point systems, zone prices, freight equalization, etc.

MARKET PROCEDURES, INCLUDING:

- Company bids on contracts for particular products; company procedures for responding to bid invitations; and
- Matters relating to actual or potential individual suppliers or customers that might have the effect of excluding them from any market or influencing the business conduct of firms toward them.

Appointment

From: Renee Lani [renee_lani@americanchemistry.com]
Sent: 10/24/2018 12:18:47 PM
To: Renee Lani [renee_lani@americanchemistry.com]; Henry, Tala [Henry.Tala@epa.gov]; Scarano, Louis [Scarano.Louis@epa.gov]; Lowit, Anna [Lowit.Anna@epa.gov]; Camacho, Iris [Camacho.Iris@epa.gov]; Irwin, William [Irwin.William@epa.gov]; Schweer, Greg [Schweer.Greg@epa.gov]; Lloyd, Matthew [Lloyd.Matthew@epa.gov]
Subject: FW: Technical Meeting with TSCA Section 5 Testing Consortium and ScitoVation
Location: 700 2nd Street NE, 20002
Start: 12/13/2018 2:00:00 PM
End: 12/13/2018 5:00:00 PM
Show Time As: Busy

FYI

-----Original Appointment-----

From: Lani, Renee <renee_lani@americanchemistry.com>
Sent: Tuesday, October 23, 2018 5:20 PM
To: Lani, Renee; Lloyd, Matthew
Subject: Technical Meeting with TSCA Section 5 Testing Consortium and ScitoVation
When: Thursday, December 13, 2018 9:00 AM-12:00 PM (UTC-05:00) Eastern Time (US & Canada).
Where: 700 2nd Street NE, 20002

Agenda to follow.

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Message

From: Ann Tveit [ann.tveit@basf.com]
Sent: 11/20/2020 2:18:28 AM
To: Henry, Tala [Henry.Tala@epa.gov]; Stedeford, Todd [Stedeford.Todd@epa.gov]; Sahar_Osman-Sypher@americanchemistry.com; Rick_Becker@americanchemistry.com
Subject: BASF comments: Surfactants Manuscript Path Forward on Peer Reviewer Comments
Attachments: Response to Reviewer Comments_11-18-20_pm_commentBASF.docx; draft manuscript general surfactants - 28 August 2020.ver.1_Rev1_11-18-20_pmat_BASF.docx

Hi All,

Hope all is well. Thanks for your efforts on this manuscript. Attached are documents with BASF comments for your consideration. One question we have – Can you please provide some insight as to the AOP vs MOA? It's changed in some places but not others and some have ?. We're not sure the basis for this and which one is the preferred. Once clarified – we may have additional comments on that topic.

Thanks again for all your efforts. Please let us know if you would like additional information.

Best Regards,
Ann

Ann Tveit Ph.D., D.A.B.T.
Toxicology Manager

Phone: +1 973 245-5527, Mobile: +19735275448, Email: ann.tveit@basf.com
Postal Address: BASF Corporation, 2B662, 100 Park Avenue, 07932 Florham Park, USA



BASF Corporation

From: Henry, Tala <Henry.Tala@epa.gov>
Sent: Wednesday, November 18, 2020 8:56 PM
To: Sahar_Osman-Sypher@americanchemistry.com; Rick_Becker@americanchemistry.com; Hayes, Michael <hayes.mp@pg.com>; Hillebold, Donna <donna.hillebold@nouryon.com>; ljovanovich@stepan.com; Keene, Athena M. <Athena.Keene@AftonChemical.com>; Kennedy, Wayne <wayne.kennedy@aftonchemical.com>; Stefan Moors <stefan.moors@basf.com>; Ogden, Julianne <Julianne_Ogden@americanchemistry.com>; Skulsky, Joseph <JSkulsky@stepan.com>; Ann Tveit <ann.tveit@basf.com>; Rose, Jane <rose.jl@pg.com>; Tremblay, Raphael <tremblay.r.2@pg.com>; Stedeford, Todd <Stedeford.Todd@epa.gov>; Salazar, Keith <Salazar.Keith@epa.gov>; Jarabek, Annie <Jarabek.Annie@epa.gov>; Irwin, William <Irwin.William@epa.gov>; amyjc@piscld.org.uk; Dr. Monita Sharma <monitas@piscld.org.uk>
Cc: Henry, Tala <Henry.Tala@epa.gov>
Subject: [EXT] RE: Surfactants Manuscript Path Forward on Peer Reviewer Comments
Importance: High

Hi all,

I apologize for forgetting to send the versions this morning, but my bad allowed Wayne & Mike to provide responses to comments and some inserts to the manuscript (both attached—with “_pm” extension). I also went through the Intro &

Risk Assessment under TSCA sections and shortened (some) – additional changes will need to be made after the MPPD modeling.

Please use these versions for further edits/etc....if you used yesterday's version, just send it my way and I will incorporate.

Thanks! Tala

Tala R. Henry, Ph.D.
Deputy Director
Office of Pollution Prevention & Toxics

T: 202-564-2959
E: henry.tala@epa.gov

-----Original Appointment-----

From: Osman-Sypher, Sahar <Sahar_Osman-Sypher@americanchemistry.com>

Sent: Friday, November 13, 2020 1:28 PM

To: Osman-Sypher, Sahar; Rick_Becker@americanchemistry.com; Hayes, Michael; Hillebold, Donna; Ijovanovich@stepan.com; Keene, Athena M.; Kennedy, Wayne; Moors, Stefan; Ogden, Julianne; Skulsky, Joseph; Tveit, Ann; Rose, Jane; Tremblay, Raphael; Stedeford, Todd; Henry, Tala; Salazar, Keith; Jarabek, Annie; Irwin, William; amyjc@piscldt.org.uk; Dr. Monita Sharma

Subject: Surfactants Manuscript Path Forward on Peer Reviewer Comments

When: Wednesday, November 18, 2020 11:00 AM-12:00 PM (UTC-05:00) Eastern Time (US & Canada).

Where: Webex

All:

Please reserve this time to discuss the response plan for the peer reviewer comments received on the surfactants manuscript.

Please use the WebEx information below to join the meeting.

https://americanchemistry.webex.com/join/rick_becker

Please Use the WebEx "Call Me" feature using a telephone; or use the "Computer Audio" with a headset.

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Thanks, Sahar

Sahar Osman-Sypher | American Chemistry Council
Director, Chemical Products and Technology Division
sahar_osman-sypher@americanchemistry.com
700 2nd Street, NE | Washington, DC | 20002
O: 202-249-6721 C: 703-362-6884
www.americanchemistry.com

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Surfactants Category: The Application of a New Approach Methodology (NAM) for Assessing Inhalation Risks under the Amended Toxic Substances Control Act

Commented [A1]: Its just TSCA now

*Tala R. Henry^{a,†}, Keith D. Salazar^{b,†}, Michael P. Hayes^c, Wayne Kennedy^d, Athena M. Keene^d,
Annie M. Jarabek^e, Stefan Moors^f, Lela Jovanovich^g, Jane L. Rose^c, Ann Tveit^f, Raphaël T.
Tremblay^c, Richard A. Becker^h, Sahar Osman-Sypher^h, Patrick D. McMullenⁱ, Scott D. Slattery^j,
William Irwin^b, Marc Odinⁱ, Julie Melia^j, Monita Sharma^k, Amy J. Clippinger^k, and Todd
Stedeford^{a,*}*

^a Office of Pollution Prevention and Toxics, Office of Chemical Safety and Pollution Prevention,
U.S. Environmental Protection Agency, Washington, DC 20460, United States

^b Risk Assessment Division, Office of Pollution Prevention and Toxics, Office of Chemical
Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, DC
20460, United States

^c Procter & Gamble, Company, Inc., St. Bernard, Ohio 45217, United States; Mason, Ohio 45040;
Temselaan 100, 1853 Strombeek-Beaver, Belgium

^d Afton Chemical Corporation, Richmond, Virginia 23219, United States

^e Health & Environmental Effects Assessment Division, Center for Public Health & Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711, United States

^f BASF Personal Care and Nutrition GmbH, Henkelstrasse 67, 40589 Duesseldorf, Germany; BASF Corporation, Florham Park, New Jersey 07932, United States

^g Stepan Company, Northfield, Illinois 60093, United States

^h American Chemistry Council, Washington, DC 20002, United States

ⁱ ScitoVation, Durham, North Carolina 27713, United States

^j SRC, Inc., North Syracuse, New York 13212, United States

^k PETA International Science Consortium Ltd., London, England

KEYWORDS: Inhalation, Surfactant, New Approach Methodologies, Lung Toxicity, Risk Assessment

ABSTRACT

Surfactants are chemical substances used in a variety of industrial operations, occupational settings, and consumer products and therefore may result in exposure and toxicity in humans.

The Toxic Substances Control Act (TSCA) requires anyone who plans to manufacture (including import) a new chemical substance for a ~~non-exempt commercial purpose~~ to provide the U.S.

Environmental Protection Agency (EPA) with a premanufacture notice (PMN) prior to

commercialization. ~~Surfactants are a class of chemical substances used in a variety of industrial operations, occupational settings, and in consumer products. Their uses in such applications~~

~~provide pathways of exposure by which potential toxicity of these compounds may occur to~~

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Abstract as Submitted = 359 Words//2,060 Characters

Abstract as Revised = 293 Words//1,709 Characters

humans. While TSCA requires submission of any existing toxicity data, it does not require generation of toxicity data for the purpose of, or prior to, submitting a PMN. TSCA requires that EPA to review the PMN to determine whether the new chemical substance presents an unreasonable risk of injury to human health or the environment. While TSCA requires submission of existing toxicity data, it does not require generation of toxicity data to for submitting a PMN and it mandates that EPA reduce or replace vertebrate animals in testing, to the extent practicable and scientifically justified. EPA therefore relies on several approaches that do not rely on *de novo* toxicity testing to assess chemical risks, including: ~~Analogue read-~~ across, in which toxicity data for a chemical of similar structure and activity ~~is~~ are used to assess the new chemical; and chemical categories (a group of chemicals whose properties are likely to be similar or follow a regular pattern as a result of mechanism, mode of toxic action or structural similarity) have been used by EPA for decades to assess new chemical substances. This investigation ~~establishes~~ was conducted to identify surfactant chemicals with toxicity data relevant for use in conducting a quantitative human health risk assessment for new surfactant substances and to define a TSCA New Chemical Category for surfactants. ~~Category~~ The category described herein identifies physical-chemical properties to determine chemical inclusion/exclusion in the category, boundaries, which are defined, toxicological analogues suitable for conducting ‘read-across’ hazard assessment (*i.e.*, hazard identification and dose-response analysis) are identified and a tiered-testing strategy aimed at using new approach methodologies (NAMs) to reduce or replace animal testing is outlined. This tiered strategy to defining and evaluating the Surfactant Category provides a pragmatic and scientifically defensible approach to facilitate for EPA’s review of PMNs for new surfactants and a strategic

testing approach to ~~collect~~ that provides the data needed to conduct or refine surfactant risk assessments while also meeting the requirements of TSCA to reduce vertebrate testing.

INTRODUCTION

Commented [A3]: SHORTEN – TALA WILL TAKE FIRST PASS

The Toxic Substances Control Act (TSCA) was amended in 2016 by the Frank R. Lautenberg Chemical Safety for the 21st Century Act (Pub. L. 114-182). The amended TSCA included substantial changes to EPA’s authorities and responsibilities, including requirements on EPA to make a determination regarding sufficiency of information, environmental releases and human exposure, and unreasonable risks. The amended TSCA also included provisions mandating EPA to “reduce and replace, to the extent practicable, [and] scientifically justified” the use of vertebrate animals in the testing of chemicals substances. Specifically, TSCA section 4(h) charges EPA with encouraging and facilitating –

- (1) the use of scientifically valid test methods and strategies that reduce or replace the use of vertebrate animals while providing information of equivalent or better scientific quality and relevance that will support regulatory decisions under TSCA;
- (2) the grouping of 2 or more chemical substances into scientifically appropriate categories in cases in which testing of a chemical substance would provide scientifically valid and useful information on other chemical substances in the category; and
- (3) the formation of industry consortia to jointly conduct testing to avoid unnecessary duplication of tests, provided that such consortia make all information from such testing available to the Administrator.

The present investigation advances each of these TSCA mandates for chemical substances characterized as surfactants.

A surfactant is any compound that reduces surface tension when dissolved in water or water solutions, or which reduces interfacial tension between two liquids, or between a liquid and a solid. [Hawley's Condensed Chemical Dictionary, R. Lewis, Van Nostrand Reinhold Co.; 1993, pg. 1108.]

Commented [A4]: Add to REFERENCES

a substance that reduces the surface tension of a liquid in which it is dissolved. They are surface-active, amphiphilic compounds that self-assemble to form micelles or aggregates above a critical concentration, referred to as the critical micelle concentration (CMC). These substances are commonly used in industrial processes, occupational settings, and in consumer products (e.g., household cleaning and products, personal care products, etc.) as detergents, wetting agents, emulsifiers, foaming agents, and dispersants. The widespread manufacture, processing and use of surfactants provides opportunities for releases and exposure to humans or environmental receptors. The inherent properties of surfactants may induce toxicity if exposures can interfere with biological surfactants or tissues. Certain surfactants are commonly used in a laboratory setting to disrupt cell membranes and denature proteins, which demonstrates the inherent hazards of surfactants. For example, sodium dodecyl sulfate (SDS; Chemical Abstracts Service Registry

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Number (CASRN) 151-21-3), a strong anionic surfactant, is used at concentrations up to 10% to disrupt cell membranes and to denature proteins, whereas octylphenoxypolyethoxyethanol (CASRN 9002-93-1), a mild nonionic surfactant, at concentrations up to 1% disrupt cell membranes, while preserving proteins for isolation [ADDIN EN.CITE

Commented [A6]: DELETE FOR BREVITY; THE TOX OF THESE ARE DISCUSSED BELOW

<EndNote><Cite><Author>Burden</Author><Year>2012</Year><RecNum>14727</RecNum>
><DisplayText>[1]</DisplayText><record><rec-number>14727</rec-number><foreign-
keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evealxfs0err5sr"

timestamp="1596017177">14727</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Burden, D.W.</author></authors></contributors><titles><title>Guide to the Disruption of Biological Samples - 2012, Version 1.1.</title><secondary-title>Random Primers</secondary-title></titles><periodical><full-title>Random Primers</full-title></periodical><pages>1-25</pages><number>12</number><dates><year>2012</year></dates><urls></urls></record></Cite></EndNote>].

Hazard concerns for surfactants historically focused on their observed environmental effects and potential toxicity to aquatic organisms based on “down the drain” releases and/or presence in effluent from wastewater treatment facilities [ADDIN EN.CITE ADDIN EN.CITE.DATA

]. [The EPA has established chemical categories for nonionic, anionic, and cationic (quaternary ammonium) surfactants based on environmental toxicity concerns [ADDIN EN.CITE

<EndNote><Cite><Author>EPA</Author><Year>2010</Year><RecNum>14729</RecNum><

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Article">17</ref-

type><contributors><authors><author>EPA</author></authors></contributors><titles><title>T

SCA New Chemicals Program (NCP) Chemical Categories</title><secondary-title>Office of

Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, D.C.

20460</secondary-title></titles><periodical><full-title>Office of Pollution Prevention and

Toxics, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-

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title></periodical><pages>157, https://www.epa.gov/sites/production/files/2014-10/documents/ncp_chemical_categories_august_2010_version_0.pdf</pages><dates><year>2010</year></dates><urls></urls></record></Cite></EndNote>]. Surfactants may pose a potential hazard to humans, depending on their use and route of exposure, because they can disrupt the normal architecture of the lipid bilayer and reduce the surface tension, thereby solubilizing cell membranes. Mucous membranes are particularly sensitive to the surface-active effects of surfactants, which have been shown to cause irritancy and injury to the eye, based on their ability to "readily penetrate the sandwiched aqueous and lipid barriers of the cornea" [ADDIN EN.CITE <EndNote><Cite><Author>Fox</Author><Year>2008</Year><RecNum>14730</RecNum><DisplayText>[4]</DisplayText><record><rec-number>14730</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evarxfs0err5sr" timestamp="1596017801">14730</key></foreign-keys><ref-type name="Book Section">5</ref-type><contributors><authors><author>Fox, D.A.</author><author>Boyes, W.K.</author></authors><secondary-authors><author>Klaassen, C.D.</author></secondary-authors></contributors><titles><title>Toxic Responses of the Ocular and Visual System</title><secondary-title>Casarett & Doull's Toxicology - The Basic Science of Poisons, Seventh Edition</secondary-title></titles><pages>665-697</pages><section>17</section><dates><year>2008</year></dates><pub-location>New York</pub-location><publisher>McGraw-Hill, Medical Publishing Division</publisher><urls></urls></record></Cite></EndNote>].

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Depending on the conditions of use, the potential for inhalation exposures to workers and/or consumers warrant consideration in quantitative risk assessments. Surfactants may cause adverse effects on mucous membranes, including the respiratory tract, and interfere with the natural pulmonary surfactants and result in reduction in the oxygen content of arterial blood due to impaired gas exchange in the pulmonary region, increases in pulmonary extravascular water volume and wet-to-dry weight ratio of the lungs, grossly visible pulmonary edema, and atelectasis [ADDIN EN.CITE ADDIN EN.CITE.DATA]. The chemical category boundary for surfactants that may have the potential to present an inhalation hazard has not been previously defined. The toxicity of surfactants by inhalation exposure can vary over several orders of magnitude, based on their chemical properties, although differences in exposure conditions are an important confounder to consider in cross category comparisons. For example, among the available data, a lowest-observed-adverse-effect concentration [LOAEC] of 5.3 mg/m³) was determined for octylphenoxypolyethoxyethanol, a nonionic surfactant, in a 14-day whole body study[ADDIN EN.CITE ADDIN EN.CITE.DATA] while a LOAEC of 0.08 mg/m³ in a 4-week nose-only study [ADDIN EN.CITE

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<EndNote><Cite><Author>EPA</Author><Year>2016</Year><RecNum>14732</RecNum><DisplayText>[10]</DisplayText><record><rec-number>14732</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evealxfds0err5sr" timestamp="1596018482">14732</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Subchronic Inhalation Toxicity Study of DDAC - Revised</title><secondary-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington,

D.C. 20460</secondary-title></titles><periodical><full-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>25</pages><volume>HQ-OPP-2006-0338-0045</volume><dates><year>2016</year></dates><urls></urls></record></Cite></EndNote>] was observed for didecyltrimethyl ammonium chloride (DDAC; CASRN 7173-51-5), a cationic surfactant and biocide.

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The objectives of the present investigation were to: (1) perform a systematic review of the literature with the aim of defining the chemical space for surfactants; (2) identify inhalation toxicity studies on surfactants that may be used to inform inhalation risk assessments; (3) describe scientifically sound new approach methodologies (NAMs) to reduce or replace animal testing; and (4) establish a tiered-testing strategy that uses NAMs to evaluate new chemistries in the Surfactant Category.

MATERIALS AND METHODS

Systematic Literature Review

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Much of the editing in the Supplemental

Two literature searches were performed, an initial search from 1950 through November 2016 and a supplemental search up to April 2018. The details of these searches, including the search strategies, search terms, search results and Population, Exposure, Comparison, and Outcome (PECO) criteria used for reviewing the relevance of the identified studies to this evaluation are provided in the Supporting Information file at “Section 1 Systematic Literature Review”. These searches were conducted with the primary objective of identifying studies that evaluated the toxicity of surfactants in the respiratory tract of humans or laboratory animals, and at the cellular

level in *in vitro* and *ex vivo* studies. In addition, these searches were used to identify potential NAMs that could inform a tiered-testing strategy for general surfactants that reduces or replaces the use of vertebrate animals in regulatory testing.

Risk Assessment Approaches under TSCA

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Risk Assessment Paradigm

~~The methods for assessing~~Assessment of risks of new chemical substances under TSCA have been developed using science-based approaches, scientific peer review, and refinement of the approaches. EPA conducts risk assessments following the four-step process articulated by the U.S. National Research Council (NRC) in 1983 [11] and reaffirmed several times since its initial release [12, 13]. This process includes hazard identification, dose-response analysis, exposure assessment, and risk characterization. Hazard assessment (also called effects assessment in some EPA guidance documents) identifies the adverse health or environmental effects, or hazards, that can be caused by exposure to a chemical substance. The dose-response analysis assesses the relationship between the exposure or dose of a chemical and the occurrence of health or environmental effects. The exposure assessment characterizes human or environmental exposures, including the magnitude, frequency, and duration, to the extent necessary and practicable within the context of the assessment. Finally, the risk characterization integrates the hazard, dose-response, and exposure components to describe the nature, and when possible, the magnitude of risks to human health and the environment.

The approaches employed for these risk assessment components, including the level of detail and complexity of quantitative aspects, may vary across different risk assessments and typically align

with specific legislative and regulatory frameworks. For example, legislative and regulatory frameworks for hazard evaluation of pesticide active ingredients, anti-microbial substances, inerts, *etc.* are described in regulations for pesticides, which include multiple and specific requirements for toxicity data. Under TSCA and its implementing regulations [ADDIN EN.CITE

<EndNote><Cite><Author>EPA</Author><Year>2020</Year><RecNum>14738</RecNum><DisplayText>[11]</DisplayText><record><rec-number>14738</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evealrxfds0err5sr" timestamp="1596019129">14738</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>40 CFR Part 720 - Premanufacture Notification</title><secondary-title>Code of Federal Regulations</secondary-title></titles><periodical><full-title>Code of Federal Regulations</full-title></periodical><pages>https://www.law.cornell.edu/cfr/text/40/part-720</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>],

companies are required to submit a PMN along with available data on: chemical identity, production volume, byproducts, use, environmental release, disposal practices, and human exposure. These submissions are required to include all existing health and environmental data in the possession or control of the submitter, parent company, or affiliates, and a description of any existing data known to or reasonably ascertainable by the submitter. However, TSCA has never included requirements for toxicity testing or generation of hazard data for new chemical substances.

Hazard Assessment

Given the lack of toxicity testing requirements under TSCA, EPA only occasionally receives hazard data for new chemical substances. ~~An analysis of toxicity data submitted to EPA from 2004 through 2012 for new chemical substances found that only about 15% of the PMN submissions included health hazard data; the majority of which was that information was for acute toxicity (e.g., 24-hour dermal toxicity study with a 14-day post-administration observation period) and irritation (e.g., 4-hour dermal irritation/corrosion with a 14-day post-administration observation period or 24-hour eye irritation/corrosion with a 21-day post-administration observation period) in laboratory animals. TSCA provides EPA with the authority to require the generation and submission of additional data when the information included with the PMN,—coupled with that available to EPA risk assessors from predictive modeling, read-across, internal archives, *etc.*—is insufficient to permit a reasoned evaluation of the health and environmental effects of a new chemical substance. However, prior to making a request for testing using vertebrate animals, EPA must first take into consideration reasonably available existing information, including toxicity information (e.g., in the scientific literature or internal archives, *etc.*; computational toxicology and bioinformatics (e.g., predictive modeling, read-across); and high-throughput screening methods and the prediction models of those methods (TSCA Section 4(h)(A)(i)-(iii)).~~

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Given the historical lack of hazard data for new chemical substances, EPA has, for decades, employed a number of approaches that do not rely on *de novo* toxicity testing. These approaches

include computational toxicology (*e.g.*, predictive models and expert systems), analogue¹ read-across wherein available toxicity data for a chemical of similar structure and activity are used to assess the new chemical substance lacking data, and chemical categories (a group of chemicals whose properties are likely to be similar or follow a regular pattern as a result of mechanism, mode of toxic action or structural similarity) [ADDIN EN.CITE ADDIN EN.CITE.DATA

]. EPA has a current chemical categories document on surfactants entitled “TSCA New Chemicals Program (NCP) Chemical Categories” [ADDIN EN.CITE

<EndNote><Cite><Author>EPA</Author><Year>2010</Year><RecNum>14729</RecNum><DisplayText>[3]</DisplayText><record><rec-number>14729</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evarxfds0err5sr" timestamp="1596017536">14729</key></foreign-keys><ref-type name="Journal Article">17</ref-

type><contributors><authors><author>EPA</author></authors></contributors><titles><title>TSCA New Chemicals Program (NCP) Chemical Categories</title><secondary-title>Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>157, https://www.epa.gov/sites/production/files/2014-10/documents/ncp_chemical_categories_august_2010_version_0.pdf</pages><dates><year>2010</year></dates><urls></urls></record></Cite></EndNote>] that includes information for

¹ In the context of this article, an analogue is a chemical substance identified based on its physicochemical and toxicological properties, as one that has undergone evaluation, as stated above, and determined to be an acceptable toxicological analogue for read across to the new chemical substance. An analogue may be directly used in read-across for informing a quantitative risk assessment on a new chemical substance.

anionic, nonionic, and cationic surfactants; however, these were previously the categories are developed and defined only for environmental toxicity considerations.

Commented [A15]: ?? This seems out of place here???

The integration of these methods with NAMs to advance testing strategies has been recognized by Dellarco *et al.* [ADDIN EN.CITE ADDIN EN.CITE.DATA] and is consistent with the vision articulated in the 2007 report by the NRC in “Toxicity Testing in the 21st Century: A Vision and Strategy” [ADDIN EN.CITE

<EndNote><Cite><Author>NRC</Author><Year>2007</Year><RecNum>14741</RecNum><DisplayText>[16]</DisplayText><record><rec-number>14741</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evealxfds0err5sr" timestamp="1596019531">14741</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>NRC</author></authors></contributors><titles><title>Toxicity Testing in the 21st Century: A Vision and a Strategy, Washington, D.C. The National Academies Press</title></titles><pages>216, DOI: <https://doi.org/10.17226/11970></pages><volume>ISBNs: Ebook: 978-0-309-13412-5; Paperback: 978-0-309-15173-3</volume><dates><year>2007</year></dates><urls></urls></record></Cite></EndNote>].

EPA defines NAMs “as a broadly descriptive reference to any technology, methodology, approach, or combination thereof that can be used to provide information on chemical hazard and risk assessment that avoids the use of intact animals” [ADDIN EN.CITE

<EndNote><Cite><Author>EPA</Author><Year>2018</Year><RecNum>14844</RecNum><DisplayText>[17]</DisplayText><record><rec-number>14844</rec-number><foreign-

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type><contributors><authors><author>EPA</author></authors></contributors><titles><title>S
trategic Plan to Promote the Development and Implementation of Alternative Test Methods
within the TSCA Program</title><secondary-title>Office of Chemical Safety and Pollution
Prevention & Office of Research and Development, U.S. Environmental Protection Agency,
Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Chemical
Safety and Pollution Prevention & Office of Research and Development, U.S.
Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>39,
https://www.epa.gov/sites/production/files/2018-06/documents/epa_alt_strat_plan_6-20-
18_clean_final.pdf</pages><volume>EPA-740-R1-
8004</volume><dates><year>2018</year></dates><urls></urls></record></Cite></EndNote>]

Dose-Response Analysis

~~In the absence of test data on new chemical substances, EPA relies on read-across methods using an analogue or a category of analogues in the absence of test data on the new chemical substance to identify hazards and conduct d~~Dose-response analysis is conducted, whether on a new chemical substance or an appropriate analogue, ~~to identify a point of departure (POD), i.e., a~~
dose or concentration that marks the beginning of a low-dose extrapolation. In the absence of test data on new chemical substances Toxicity data for analogues are used to identify a POD, such as a no observed adverse effect (concentration) level (NOAE(C)L) or lowest observed adverse effect (concentration) level (LOAE(C)L, for assessing risks of the new chemical substance. This

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POD can also be the lower bound on dose (or concentration) for an estimated incidence or a change in response level calculated by a dose-response model such as those available in EPA's benchmark dose software (BMDS), *e.g.*, the BMCL for an observed incidence or change in level of response [ADDIN EN.CITE

<EndNote><Cite><Author>EPA</Author><Year>2012</Year><RecNum>14744</RecNum><

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enchmark Dose Technical Guidance</title><secondary-title>Risk Assessment Forum, U.S.

Environmental Protection Agency, Washington, D.C. 20460</secondary-

title></titles><periodical><full-title>Risk Assessment Forum, U.S. Environmental Protection

Agency, Washington, D.C. 20460</full-title></periodical><pages>99,

[\[https://www.epa.gov/sites/production/files/2015-\]\(https://www.epa.gov/sites/production/files/2015-01/documents/benchmark_dose_guidance.pdf\)](https://www.epa.gov/sites/production/files/2015-</p></div><div data-bbox=)

[01/documents/benchmark_dose_guidance.pdf](https://www.epa.gov/sites/production/files/2015-01/documents/benchmark_dose_guidance.pdf)</pages><volume>EPA/100/R-

12/001</volume><dates><year>2012</year></dates><urls></urls></record></Cite></EndNote

>]. EPA's current chemical categories document on surfactants entitled "TSCA New Chemicals Program (NCP) Chemical Categories" [ADDIN EN.CITE

<EndNote><Cite><Author>EPA</Author><Year>2010</Year><RecNum>14729</RecNum><

DisplayText>[3]</DisplayText><record><rec-number>14729</rec-number><foreign-

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SCA New Chemicals Program (NCP) Chemical Categories</title><secondary-title>Office of
Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, D.C.
20460</secondary-title></titles><periodical><full-title>Office of Pollution Prevention and
Toxics, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-
title></periodical><pages>157, [https://www.epa.gov/sites/production/files/2014-
10/documents/ncp_chemical_categories_august_2010_version_0.pdf](https://www.epa.gov/sites/production/files/2014-10/documents/ncp_chemical_categories_august_2010_version_0.pdf)</pages><dates><year>201
0</year></dates><urls></urls></record></Cite></EndNote>] includes information for anionic,
nonionic, and cationic surfactants; however, these were previously developed and defined only
on environmental toxicity considerations.

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EPA's has also developed guidance to improve the science underlying the animal-to-human
uncertainty factor (UF_A), which and provides generalized procedures for deriving dosimetric
adjustment factors (DAFs) to perform interspecies extrapolation [ADDIN EN.CITE

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<EndNote><Cite><Author>EPA</Author><Year>2002</Year><RecNum>14743</RecNum><
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Article">17</ref-

type<<contributors><authors><author>EPA</author></authors></contributors><titles><title>A
Review of the Reference Dose and Reference Concentration Processes</title><secondary-
title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C.

20460</secondary-title></titles><periodical><full-title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>192, <https://www.epa.gov/sites/production/files/2014-12/documents/rfd-final.pdf></pages><volume>EPA/630/P-02/002F</volume><dates><year>2002</year></dates><urls></urls></record></Cite><Cite><Author>EPA</Author><Year>1994</Year><RecNum>14746</RecNum><record><rec-number>14746</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596021628">14746</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry</title><secondary-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</secondary-title></titles><periodical><full-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</full-title></periodical><pages>389, https://www.epa.gov/sites/production/files/2014-11/documents/rfc_methodology.pdf</pages><volume>EPA/600/8-90/066F</volume><dates><year>1994</year></dates><urls></urls></record></Cite></EndNote>

e>] is also used in dose-response analysis. Application of DAFs to the animal airborne exposure values yields estimates of the concentration that would result in the same concentration to humans, that is, the human equivalent concentration (HEC). Application of a DAF in the calculation of an HEC is considered to address the toxicokinetic (TK; *i.e.*, absorption, distribution, metabolism, and excretion) aspects, but not the toxicodynamic (TD; *i.e.*, mode of

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action) component, of the animal-to-human uncertainty factor (UF_{TH}) (i.e., to estimate from

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animal exposure information the human exposure scenario that would result in the same dose as achieved in the animal to a given target tissue) [ADDIN EN.CITE

<EndNote><Cite><Author>EPA</Author><Year>2002</Year><RecNum>14743</RecNum><

DisplayText>[19]</DisplayText><record><rec-number>14743</rec-number><foreign-

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Article">17</ref-

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20460</secondary-title></titles><periodical><full-title>Risk Assessment Forum, U.S.

Environmental Protection Agency, Washington, D.C. 20460</full-

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12/documents/rfd-final.pdf</pages><volume>EPA/630/P-

02/002F</volume><dates><year>2002</year></dates><urls></urls></record></Cite></EndNot

e>]. This operational derivation of a DAF involves the use of species-specific physiologic and

anatomic factors relevant to the form of pollutant (e.g., particle, reactive gas, or volatile organic

compound) coupled with consideration of the location and type of toxic response. These factors

are all employed in determining the appropriate DAF. For HECs, DAFs are applied to the

“duration-adjusted” concentration to which the animals were exposed (e.g., to a weekly average

based on number of h/d and d/w).

For interspecies extrapolation of particle exposures, the Regional Deposited Dose Ratio (RDDR) model developed by EPA can be used to derive a DAF. The RDDR is the ratio of the deposited dose in a respiratory tract region (r) for the laboratory animal species of interest (RDD_A) to that of humans (RDD_H) [ADDIN EN.CITE

<EndNote><Cite><Author>EPA</Author><Year>1994</Year><RecNum>14746</RecNum><

DisplayText>[20]</DisplayText><record><rec-number>14746</rec-number><foreign-

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Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation

Dosimetry</title><secondary-title>Office of Research and Development, U.S. Environmental

Protection Agency, Research Triangle Park, NC</secondary-title></titles><periodical><full-

title>Office of Research and Development, U.S. Environmental Protection Agency, Research

Triangle Park, NC</full-title></periodical><pages>389,

[\[11/documents/rfc_methodology.pdf\]\(https://www.epa.gov/sites/production/files/2014-11/documents/rfc_methodology.pdf\)</pages><volume>EPA/600/8-](https://www.epa.gov/sites/production/files/2014-</p>
</div>
<div data-bbox=)

90/066F</volume><dates><year>1994</year></dates><urls></urls></record></Cite></EndNot

e>]. EPA's RDDR model allows calculation of RDDR estimates in various regions of the

respiratory tract for animals versus humans (*i.e.*, extra-thoracic [ET], tracheobronchial [TB],

pulmonary [PU], thoracic [TH], total respiratory tract [RT] and extra-respiratory [ER] regions).

The RDDR calculation is based on the characteristics of the aerosol tested in the inhalation study

(*i.e.*, the Median Mass Aerodynamic Diameter or MMAD, Geometric Standard Deviation or

GSD, and density), and species-specific parameters for both animals and humans including ventilation rates and regional surface areas of the respiratory tract. The RDDR selected as the DAF is informed by the effects (clinical signs, tissue effects, biochemical changes) observed in the animal toxicity study and the aerosol characteristics in the inhalation study. The DAF is then applied to the duration-adjusted POD to arrive at the HEC of the POD (POD_{HEC}). The EPA's RDDR model was used herein to calculate HEC values from the aerosol exposures to laboratory animals available for each of the surfactant classes.

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After an analogue(s) is identified, the strengths, limitations, and uncertainties associated with the use of the ~~substance~~analogue(s) to predict the hazards and POD for the new chemical substance under evaluation are considered when deriving a benchmark margin of exposure (MOE). The benchmark MOE is the result of multiplying all relevant UFs to account for: (1) the variation in susceptibility among the members of the human population (*i.e.*, inter- individual or intraspecies variability); (2) the extrapolation from animal data to humans (*i.e.*, interspecies extrapolation); (3) the extrapolation from data in a study with less- than- lifetime exposure (*i.e.*, extrapolating from sub-chronic to chronic exposure); (4) the extrapolation from a LOAEL to a NOAEL [ADDIN EN.CITE

<EndNote><Cite><Author>EPA</Author><Year>2002</Year><RecNum>14743</RecNum><DisplayText>[19, 21]</DisplayText><record><rec-number>14743</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evealxfs0err5sr" timestamp="1596019884">14743</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>A

Review of the Reference Dose and Reference Concentration Processes</title><secondary-
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Environmental Protection Agency, Washington, D.C. 20460</full-
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02/002F</volume><dates><year>2002</year></dates><urls></urls></record></Cite><Cite><
Author>EPA</Author><Year>2014</Year><RecNum>14742</RecNum><record><rec-
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uidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for
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20460</secondary-title></titles><periodical><full-title>Office of the Science Advisor, Risk
Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-
title></periodical><pages>109, <https://www.epa.gov/sites/production/files/2015-01/documents/ddef-final.pdf></pages><volume>EPA/R-
14/002F</volume><dates><year>2014</year></dates><urls></urls></record></Cite></EndNot
e>]. EPA prefers using existing information to develop data-derived extrapolation factors
(DDEFs) or chemical specific adjustment factors (CSAFs) rather than relying on default values [
ADDIN EN.CITE

<EndNote><Cite><Author>EPA</Author><Year>2014</Year><RecNum>14742</RecNum><DisplayText>[21]</DisplayText><record><rec-number>14742</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evealxfs0err5sr" timestamp="1596019768">14742</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation</title><secondary-title>Office of the Science Advisor, Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of the Science Advisor, Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>109, <https://www.epa.gov/sites/production/files/2015-01/documents/ddef-final.pdf></pages><volume>EPA/R-14/002F</volume><dates><year>2014</year></dates><urls></urls></record></Cite></EndNote>]. This investigation includes several approaches to derive DDEFs for use in assessing new surfactant chemical substances.

Exposure Assessment

Commented [A19]: EPA TO REVISE IN CONTEXT OF MPPD: ANNIE, TALA, TODD W/Keith & William

In assessing new chemical substances, generally new chemical substances do not have occupational exposure monitoring data or consumer exposure data; therefore, EPA typically evaluates occupational exposures first, given that these represent the highest exposure estimates. Therefore, this evaluation focused on occupational exposures, recognizing that consumer exposures would also be considered, if applicable. EPA develops exposure estimates for workers using the Chemical Screening Tool for Exposures and Environmental Releases (ChemSTEER)

model. ChemSTEER estimates exposure as daily acute potential dose rates (PDRs) or lifetime average daily doses (LADDs). The PDR represents average exposure over an 8-hour workday, whereas the LADD estimates long-term exposures to the chemical substance and is averaged over a lifetime exposure of 75 years. The PDR, an initial conservative exposure estimate, is considered to be the more appropriate dose-metric for estimating risks to surfactants because surfactants are surface-active at the point of exposure and effects in the respiratory tract occur rapidly following exposure. This assumes that neither the chemical nor its damage accumulate or distribute to systemic compartments. For chemical substances used in a liquid, mist, or aerosol form, the general default PDR values are 1.875 mg/kg-bw/day for inhalable aerosols or 0.625 mg/kg-bw/day for respirable aerosols as shown in [REF _Ref46930162 \h *

MERGEFORMAT] [ADDIN EN.CITE

<EndNote><Cite><Author>EPA</Author><Year>2015</Year><RecNum>14745</RecNum><

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Article">17</ref-

type><contributors><authors><author>EPA</author></authors></contributors><titles><title>C

hemSTEER User Guide, Chemical Screening Tool for Exposures and Environmental

Releases</title><secondary-title>Office of Pollution Prevention and Toxics, U.S. Environmental

Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-

title>Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency,

Washington, D.C. 20460</full-title></periodical><pages>403,

<https://www.epa.gov/sites/production/files/2015->

05/documents/user_guide.pdf</pages><dates><year>2015</year></dates><urls></urls></record></Cite></EndNote>].

Table [SEQ Table * ARABIC]. Default values used for calculating the daily acute potential dose rate (PDR).

Commented [A20]: EPA NEEDS TO ADDRESS IN CONTEXT OF MPPD – ANNIE, TODD, TALA, KEITH

Description	Equation	Description	Equation ^a	Defaults	Units
PDR (mg/kg-bw/day)	I/BW	Inhalation PDR (I)	$C_m \times b \times h$, where C_m is the mass concentration of chemical in air, b is the volumetric inhalation rate ($0 < b \leq 7.9$), and h is the exposure duration ($0 \leq h \leq 24$)	$C_m = 15 \text{ mg/m}^3$ $b = 1.25 \text{ m}^3/\text{hr}$ $h = 8 \text{ hours/day}$	mg/day
		Body weight (BW)	BW ($0 \leq BW$)	80 kg-bw	kg-bw

^a C_m may also be adjusted for the mass concentration of the chemical with a permissible exposure limit (PEL) in air (based on the U.S.

Occupational Safety and Health Administration [OSHA] PEL – time-weighted average [TWA]; where: K_{Ck} = the mass concentration limit of total particulate in air (mg/m^3) with a default of 15 mg/m^3 for inhalable and 5 mg/m^3 for respirable, Y_s = the weight fraction of chemical in particulate ($0 < Y_s \leq 1$), Y_{pel} = the weight fraction of chemical or metal in particulate with a known PEL ($0 < Y_{pel} \leq 1$) using the following equation: $C_m = K_{Ck} \times Y_s / Y_{pel}$

The PDR is calculated using an exposure regimen for a default worker of 8 hours/day and 5 days/week, unless chemical-specific manufacture, processing or use information are provided in the PMN. The exposure conditions in laboratory animal studies often do not reflect occupational exposure scenarios; therefore, a duration adjustment and a DAF (*i.e.*, RDDR value) are applied to the POD to derive HECs for exposed human populations according to Agency methods [ADDIN EN.CITE

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<EndNote><Cite><Author>EPA</Author><Year>1994</Year><RecNum>14746</RecNum><DisplayText>[20]</DisplayText><record><rec-number>14746</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evarxfds0err5sr" timestamp="1596021628">14746</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry</title><secondary-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</secondary-title></titles><periodical><full-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</full-title></periodical><pages>389, https://www.epa.gov/sites/production/files/2014-11/documents/rfc_methodology.pdf</pages><volume>EPA/600/8-90/066F</volume><dates><year>1994</year></dates><urls></urls></record></Cite></EndNote>]. Therefore, the interspecies extrapolation is performed using particle deposition models that adjust for the aerodynamics of the given particles in the different airway architecture between the

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species and using species-specific physiologic parameters such as ventilation. The occupational exposure is characterized with human ventilation rates during exertion (work) and exposure durations appropriate to the specific occupational setting and chemical use scenario.

Risk Characterization

Risk characterization is the final, integrative step of risk assessment. EPA's Risk Characterization Policy defines risk characterization as the integration of information from the hazard and exposure components of the risk assessment into an overall conclusion about the existence (or lack of) risk that is complete, informative, and useful for decision making. The risk characterization conveys the risk assessor's judgment as to the nature and existence of (or lack of) human health or ecological risks [ADDIN EN.CITE

<EndNote><Cite><Author>EPA</Author><Year>2000</Year><RecNum>14747</RecNum><DisplayText>[23]</DisplayText><record><rec-number>14747</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596021806">14747</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Risk Characterization</title><secondary-title>Office of Science Policy, Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Science Policy, Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>189, https://nepis.epa.gov/Exe/ZyPDF.cgi/40000006.PDF?Dockkey=40000006.PDF</pages><volume

>EPA 100-B-00-

002</volume><dates><year>2000</year></dates><urls></urls></record></Cite></EndNote>].

It is recognized that As described in EPA's Risk Characterization Handbook "Risk characterization at EPA assumes different levels of complexity depending on the nature of the risk assessment being characterized and the level of information contained in each risk characterization varies according to the type of assessment for which the characterization is written and the audience for which the characterization is intended."

Under TSCA section 5, EPA must determine whether a chemical substance presents an unreasonable risk of injury to health or the environment under the conditions of use. EPA generally uses an MOE approach to characterize risks of new chemical substances as a starting point to estimate non-cancer risks for acute and chronic exposures. The MOE approach is a widely recognized point estimate method and provides a risk profile for different non-cancer health effects and different exposure scenarios. The MOE is the HEC derived from a POD for a health endpoint (from hazard assessment) divided by the exposure concentration for the scenario of concern (from exposure assessment). The calculated MOE is compared with a benchmark MOE to evaluate whether there is an adequate margin between human exposure estimates and the HEC. When the MOE is less than the benchmark MOE, there is a possibility of human health risks. On the other hand, risks are not expected negligible concerns would be expected if the MOE exceeds the benchmark MOE. The MOE approach is a widely recognized point estimate method and provides a risk profile for different non-cancer health effects and different exposure scenarios.

In summary, in developing a risk assessment for new chemical substances under TSCA section 5, EPA uses empirical data or analogues, to identify a POD(s) and to develop an exposure estimate for use in the evaluation. The hazard assessment in combination with the exposure assessment is used to calculate an MOE, which is compared to the benchmark MOE to identify potential risks. The risk characterization is used to inform the TSCA “unreasonable risk” determination.

RESULTS AND DISCUSSION

Literature Search and Screening Results

Commented [A21]: AMY/MONITA REVISE

An initial search of PubMed identified 594 articles that were subjected to title and abstract screening. Of these articles, 551 did not meet the PECO criteria, whereas 43 met the PECO criteria and were selected for full text review. An additional 17 articles that met the PECO criteria were identified through additional search strategies, screening gray literature, references for other types of chemical substances, *etc.*, and were included for full text review. Of the 60 articles evaluated through full text screening, 25 were identified as relevant and carried forward in the present evaluation, whereas the remaining 35 articles were excluded because they lacked relevant information on respiratory tract effects or presented inconclusive epidemiology findings. In the supplemental literature search of PubMed and Embase, 1247 articles (combined) were identified. Following title and abstract screening, 1217 of these articles were excluded because they did not meet the PECO criteria, whereas 25 met the PECO criteria and were selected for full text review. An additional 10 studies that met the PECO criteria were found by additional hand searching) and were selected for full text screening, which resulted in 35 articles that were

identified for review; ten articles were deemed irrelevant and excluded. A total of 25 articles were identified from both searches, one was excluded because it was in a foreign language and the remaining 24 articles are summarized in Table 8 in the Supporting Information file at “Section 1 Systematic Literature Review”.

The information identified in the systematic review was used to determine Category Boundaries and subcategories, to summarize the health effects of surfactants under the section on Hazard Identification, and to identify potential NAMs for use in the Tiered-Testing Strategies.

Category Boundaries

The following structural and functional criteria (hereinafter referred to as the “Surfactant Criteria”) are used to distinguish chemical substances, which include polymers and UVCB substances,² intended for use as surfactants from other amphiphilic compounds (*e.g.*, ethanol) [ADDIN EN.CITE ADDIN EN.CITE.DATA]:

1. A substance which has surface-active properties, and which consists of one or more hydrophilic and one or more hydrophobic groups;
2. The substance is capable of reducing the surface tension between air and water to 45 milliNewtons/meter (mN/m) or below at a test concentration of 0.5 wt% in water and a temperature of 20°C (*Cf.* Pure water has a surface tension of 72.8 mN/m at 20°C); and

² Chemical Substances of Unknown or Variable Composition, Complex Reaction Products and Biological Materials (UVCB Substance)

3. The substance self-associates in water to form micellar or vesicular aggregates at a concentration of 0.5 wt% or less (as measured using a standard method).

Commented [A22]: Reviewer 2: How is this measured? WAYNE/MIKE?
ADD A BRIEF DESCRIPTION/ PARENTHETICAL AND REFERENC HERE

Commented [A23R22]: Based on the response from Wayne/Mike...there are many methods so wont add specifics here; rather, just respond to the comments

The Surfactants Category is further defined into three general subcategories including nonionic, anionic, and cationic substances. Amphoteric chemical substances that meet the Surfactant Criteria would also be included within these subcategories (*i.e.*, anionic and cationic surfactants), depending on their pH. Lung lining fluids are near neutral pH, with various measurements ranging from 6.6 to 7.1 [ADDIN EN.CITE ADDIN EN.CITE.DATA]. The pKa for each component of an amphoteric surfactant should be evaluated within this pH range and the assessment should be conducted on the predominant components. The non-ionized fraction for acids/bases is calculated as follows:

$$\text{Acids Fraction}_{\text{non-ionized}} = 1 / (1 + 10^{\text{pH}-\text{pKa}})$$

$$\text{Bases Fraction}_{\text{non-ionized}} = 1 / (1 + 10^{\text{pKa}-\text{pH}})$$

Where the pH represents the physiological pH in the lung lining fluid (*i.e.*, 6.6 to 7.1), and the pKa represents the value for the respective component (*e.g.*, carboxylic acid or amine).

Nonionic surfactants are identified as any neutral chemical substance that meets the Surfactant Criteria. Common nonionic surfactants include alkylphenol chemical substances with one or more ethoxylate (EO) unit as well as linear and branched alcohol chemical substances with one or more EO units. Examples of For example, octylphenoxypolyethoxyethanol, a common

nonionic surfactants and the range of corresponding surface tension measurements associated with them octylphenol EO surfactant, and Polysorbate 80 (or Tween 80; CASRN: 9005-65-6); another nonionic alkylphenol ethoxylate with increased alkyl chain length and number of EO units, are shown in [REF _Ref47613375 \h * MERGEFORMAT]. The surface tensions of octylphenoxypolyethoxyethanol and Polysorbate 80 range from 30-31 mN/m to 37.96 mN/m, respectively ([REF _Ref47613375 \h * MERGEFORMAT])-[ADDIN EN.CITE

<EndNote><Cite><Author>Kothekar</Author><Year>2007</Year><RecNum>14758</RecNum><DisplayText>[30]</DisplayText><record><rec-number>14758</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evealrfs0err5sr" timestamp="1596025228">14758</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Kothekar, S.C.</author><author>Ware, A.M.</author><author>Waghmare, J.T.</author><author>Momin, S.A.</author></authors></contributors><titles><title>Comparative Analysis of the Properties of Tween-20, Tween-60, Tween-80, Arlacel-60, and Arlacel-80</title><secondary-title>Journal of Dispersion Science and Technology</secondary-title></titles><periodical><full-title>Journal of Dispersion Science and Technology</full-title></periodical><pages>477-484, https://www.tandfonline.com/doi/abs/10.1080/01932690601108045</pages><volume>28</volume><number>3</number><dates><year>2007</year></dates><urls></urls></record></Cite></EndNote>].

Commented [A24]: To shorten, could cut out the EXAMPLES in each paragraph and just refer to TABLE 2 ... see edits

Anionic surfactants are identified as any chemical substance with a net negative charge that meets the Surfactant Criteria (*e.g.*, alkyl sulfonates, alkylbenzene sulfonates, alkylether sulfates,

alkyl silicic acids, alkyl phosphates, alkyl carboxylic acids, or combinations of these anionic groups). An example anionic surfactant, SDS, has a reported surface tension of 35 mN/m ([REF _Ref47613375 \h * MERGEFORMAT]).

Commented [A25]: If we do shortening above and below same should be done here.

Cationic surfactants are identified as any chemical substance with a net positive charge that meets the Surfactant Criteria (e.g., alkylammonium chlorides and benzalkonium chlorides). Benzalkonium chloride (BAC; CASRN 8004-54-5) and didecyl dimethyl ammonium chloride (DDAC; CASRN 7173-51-5) are representative members of this subcategory, with surface tensions of 37 mN/m and 25.82 mN/m ([REF _Ref47613375 \h * MERGEFORMAT]), respectively are provided in Table 2. It is noted that BAC and DDAC also possess biocidal properties.

Typical commercial surfactants (nonionic, anionic, and cationic) are non-volatile³ liquids or solids. This category framework focuses on exposure *via* aerosol forms (*i.e.*, both airborne droplets and solid particles, including the hygroscopic variety) of these surfactants. While the commercial use of volatile surfactants is unlikely, it should be noted that this framework is not applicable to any substances that qualify as surfactants and are volatile under the conditions of use.

³ Volatility is considered as part of the ChemSTEER modeling, wherein a vapor pressure of 1.3×10^{-04} kPa is the cutoff for gases/vapors.

Table [SEQ Table * ARABIC]. Example Chemicals that Meet “Surfactant Criteria” and Nonionic, Anionic and Cationic Subcategorization.

Nonionic Surfactants					
Chemical Name in Text	Other Relevant Names	Criteria 1		Criteria 2	Criteria 3
		Hydrophobic group(s)	Hydrophilic group(s)	Surface Tension	Critical Micelle Concentration (CMC)
formaldehyde, polymer with oxirane and 4-(1,1,3,3-tetramethylbutyl)-phenol Defomaire Alevaire Tyloxapol CASRN: 25301-02-4	CAS Name: formaldehyde, polymer with oxirane and 4-(1,1,3,3-tetramethylbutyl)-phenol	multiple octyl phenol groups	multiple polyoxyethylene (9) units	~37 mN/m at 5 g/L (0.5 wt%) and 25°C* [ADDIN EN.CITE <EndNote><Cite><Author>Schott</Author><Year>1998</Year><RecNum>14754</RecNum><DisplayText>[31]</DisplayText><record><rec-number>14754</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5eearxfs0err5sr" timestamp="1596024000">14754</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Schott	0.038 g/L or 0.0038 wt% [ADDIN EN.CITE <EndNote><Cite><Author>Schott</Author><Year>1998</Year><RecNum>14754</RecNum><DisplayText>[31]</DisplayText><record><rec-number>14754</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5eearxfs0err5sr" timestamp="1596024000">14754</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Sch

				H.</author></authors> </contributors><auth- address>School of Pharmacy, Temple University, Philadelphia, Pennsylvania, 19140</auth- address><titles><title> Comparing the Surface Chemical Properties and the Effect of Salts on the Cloud Point of a Conventional Nonionic Surfactant, Octoxynol 9 (Triton X-100), and of Its Oligomer, Tyloxapol (Triton WR- 1339)</title><seconda- ry-title>J Colloid Interface Sci</secondary- title><alt-title>Journal of colloid and interface science</alt- title></titles><periodic- al><full-title>Journal of colloid and interface science</full- title><abbr-1>J Colloid Interface Sci</abbr-	ott, H.</author></authors> </contributors><aut h-address>School of Pharmacy, Temple University, Philadelphia, Pennsylvania, 19140</auth- address><titles><title> >Comparing the Surface Chemical Properties and the Effect of Salts on the Cloud Point of a Conventional Nonionic Surfactant, Octoxynol 9 (Triton X-100), and of Its Oligomer, Tyloxapol (Triton WR- 1339)</title><second ary-title>J Colloid Interface Sci</secondary- title><alt- title>Journal of colloid and interface science</alt- title></titles><period ical><full- title>Journal of colloid and interface
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octylphenoxypolyethoxyethanol CASRN: 9002-93-1	Triton X-100 Octoxynol 9 octylphenol ethoxylate CAS Name: poly(oxy-1,2-ethanediyl), .alpha.-[4-1,1,3,3-tetramethylbutyl)phenyl]-.omega.-hydroxy	octylphenol group	polyoxyethylene (9) unit	~30.5 mN/m at 5 g/L (0.5 wt%) and 25°C* [ADDIN EN.CITE<EndNote><Cite><Author>Schott</Author><Year>1998</Year><RecNum>14754</RecNum><DisplayText>[31]</DisplayText><record><rec-number>14754</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5eearxfs0err5sr" timestamp="1596024000">14754</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Schott, H.</author></authors>	0.17 g/L or 0.017 wt% [ADDIN EN.CITE<EndNote><Cite><Author>Schott</Author><Year>1998</Year><RecNum>14754</RecNum><DisplayText>[31]</DisplayText><record><rec-number>14754</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5eearxfs0err5sr" timestamp="1596024000">14754</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Schott,

				</contributors><auth-address>School of Pharmacy, Temple University, Philadelphia, Pennsylvania, 19140</auth-address><titles><title>Comparing the Surface Chemical Properties and the Effect of Salts on the Cloud Point of a Conventional Nonionic Surfactant, Octoxynol 9 (Triton X-100), and of Its Oligomer, Tyloxapol (Triton WR-1339)</title><secondary-title>J Colloid Interface Sci</secondary-title><alt-title>Journal of colloid and interface science</alt-title></titles><periodical><full-title>Journal of colloid and interface science</full-title><abbr-1>J Colloid Interface Sci</abbr-1></periodical><alt-	H.</author></authors></contributors><auth-address>School of Pharmacy, Temple University, Philadelphia, Pennsylvania, 19140</auth-address><titles><title>>Comparing the Surface Chemical Properties and the Effect of Salts on the Cloud Point of a Conventional Nonionic Surfactant, Octoxynol 9 (Triton X-100), and of Its Oligomer, Tyloxapol (Triton WR-1339)</title><secondary-title>J Colloid Interface Sci</secondary-title><alt-title><alt-title>Journal of colloid and interface science</alt-title></titles><periodical><full-title>Journal of colloid and interface science</full-
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polyoxyethylene-10-oleyl ether (C _{18:1} E ₁₀) CASRN: 9004-98-2	oleyl ethoxylate CAS Name: poly(oxy-1,2-ethanediyl), .alpha.-(9Z)-9-octadecen-1-yl-.omega.-hydroxy	oleyl group	polyoxyethylene (10) unit	35.17 mN/m at 4×10 ⁻⁵ M (0.028 wt%) and 25°C* [ADDIN EN.CITE <EndNote><Cite><Author>Liu</Author><Year>2006</Year><RecNum>14761</RecNum><DisplayText>[32]</DisplayText><record><record-number>14761</record-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre00azr5eearxfds0err5sr" timestamp="1596025582">14761</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Liu,	4×10 ⁻⁵ M or 0.028 wt % at 25°C [ADDIN EN.CITE <EndNote><Cite><Author>Liu</Author><Year>2006</Year><RecNum>14761</RecNum><DisplayText>[32]</DisplayText><record><record-number>14761</record-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre00azr5eearxfds0err5sr" timestamp="1596025582">14761</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Liu,

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polyoxyethylene-10-dodecyl ether (C ₁₂ E ₁₀) CASRN: 9002-92-0	polyoxyethylene (10) lauryl ether CAS Name: poly(oxy-1,2-ethanediyl),-.alpha.-dodecyl-.omega.-	dodecyl group	polyoxyethylene (10) unit	C12E9: 36 mN/m (concentration not reported) at 23°C* C12E12: 32 mN/m (concentration not reported) at 23°C* [ADDIN EN.CITE <EndNote><Cite><Author>Rosen</Author><Year>1989</Year><RecNum>14763</RecNum><DisplayText>[33]</DisplayText><record><rec-number>14763</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evealrdfs0err5sr" timestamp="1596026543">14763</key></foreign-keys><ref-type name="Edited	12.7×10 ⁻⁶ M or 0.0008 wt% at 30°C [ADDIN EN.CITE <EndNote><Cite><Author>Sulthana</Author><Year>2000</Year><RecNum>14762</RecNum><DisplayText>[34]</DisplayText><record><rec-number>14762</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evealrdfs0err5sr" timestamp="1596025808">14762</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Sulthana,

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					<p><volume>16</volume><number>3</number><dates><year>2000</year></dates><urls></urls></record></Cite></EndNote>]</p> <p>Also, C12E9 at 1×10^{-6} M at 23°C and C12E12 at 1.4×10^{-6} M at 23°C [ADDIN EN.CITE <EndNote><Cite><Author>Rosen</Author><Year>1989</Year><RecNum>14763</RecNum><DisplayText>[33]</DisplayText><record><rec-number>14763</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evarxfds0err5sr" timestamp="1596026543">14763</key></foreign-keys><ref-type name="Edited Book">28</ref-type><contributors><authors><author>Rosen,</p>
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					M.J.</author></authors></contributors><titles><title>Surfactants and interfacial phenomena</title></titles><pages>431,</pages><dates><year>1989</year></dates><pub-location>New York</pub-location><publisher>John Wiley & Sons, Inc.</publisher><urls></urls></record></Cite></EndNote>]
Polysorbate 20 (Tween 20) CASRN: 9005-64-5	polyoxyethylene (20) sorbitan monolaurate CAS Name: sorbitan, monododecanoate, poly(oxy-1,2-ethanediyl) derivs.	dodecanoyl group	sorbitan polyoxyethylene (20) unit	38 mN/m at 8.04×10^{-5} M (0.001 wt%) and 21°C* [ADDIN EN.CITE <EndNote><Cite><Author>Kim</Author><Year>2001</Year><RecNum>14756</RecNum><DisplayText>[35]</DisplayText><record><rec-number>14756</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0	8.04×10^{-5} M or 0.001 wt% at 21°C [ADDIN EN.CITE <EndNote><Cite><Author>Kim</Author><Year>2001</Year><RecNum>14756</RecNum><DisplayText>[35]</DisplayText><record><rec-number>14756</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0

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Polysorbate 80 (Tween 80) CASRN: 9005-65-6	polyoxyethylene (20) sorbitan monooleate CAS Name: sorbitan, mono-(9Z)-9-octadecenoate, poly(oxy-1,2-ethanediyl) derivs.	octadecenoyl group	sorbitan polyoxyethylene (20) unit	37.96 mN/m at 5 g/L (0.5 wt%) and 30°C [ADDIN EN.CITE<EndNote><Cite><Author>Kothekar</Author><Year>2007</Year><RecNum>14758</RecNum><DisplayText>[30]</DisplayText><record><rec-number>14758</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5eearxfds0err5sr" timestamp="1596025228">14758</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Kothekar, S.C.</author><author>Ware,	1.5×10 ⁻⁵ M or 0.002 wt% at 25°C [ADDIN EN.CITE<EndNote><Cite><Author>Mahmood</Author><Year>2013</Year><RecNum>14757</RecNum><DisplayText>[36]</DisplayText><record><rec-number>14757</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5eearxfds0err5sr" timestamp="1596024783">14757</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Mahmood, M.E.</author><author>

				<p>A.M.</author><author>Waghmare, J.T.</author><author>Momin, S.A.</author></author></contributors><titles><title>Comparative Analysis of the Properties of Tween-20, Tween-60, Tween-80, Arlacel-60, and Arlacel-80</title><secondary-title>Journal of Dispersion Science and Technology</secondary-</p> <p>title></titles><periodical><full-title>Journal of Dispersion Science and Technology</full-title></periodical><pages>477-484, https://www.tandfonline.com/doi/abs/10.1080/01932690601108045</pages><volume>28</volume><number>3</number><dates><year>2007</year></dates><urls></urls></record></Cite></EndNote>]</p>	<p>r>Al-Koofee, D.A.F.</author></author></contributors><titles><title>Effect of Temperature Changes on Critical Micelle Concentration for Tween Series Surfactants</title><secondary-title>Global Journal of Science Frontier Research Chemistry</secondary-</p> <p>title></titles><periodical><full-title>Global Journal of Science Frontier Research Chemistry</full-title></periodical><pages>5, https://journalofscience.org/index.php/GJSFR/article/view/816/681</pages><volume>13(B)</volume><number>4</number><dates><year>2013</year></dates><urls></urls></record></Cite></EndNote>]</p>
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				rd></Cite></EndNote>]	
Poloxamer 188 CASRN: 691397-13-4	CAS Name: oxirane, 2-methyl-, polymer with oxirane, triblock	polyoxypropylene (27) unit	two polyoxyethylene (80) units	~42-44 mN/m at ~0.5 wt% and 36°C [ADDIN EN.CITE ADDIN EN.CITE.DATA]	4.8×10 ⁻⁴ M or 0.4 wt% at 37°C [ADDIN EN.CITE ADDIN EN.CITE.DATA]
N,N-dimethyl-dodecylamine-N-oxide (C ₁₂ AO)*** CASRN: 1643-20-5	lauryl dimethylamine oxide CAS Name: 1-dodecanamine, N,N-dimethyl-, N-oxide	dodecyl group	amine oxide unit	34.1 mN/m at 1 g/L (0.1 wt%) and 20°C [ADDIN EN.CITE <EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14772</RecNum><DisplayText>[39]</DisplayText><record><rec-number>14772</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596028055">14772</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Registration Dossier</author></author></aut	1.7×10 ⁻³ M or 0.039 wt% [ADDIN EN.CITE <EndNote><Cite><Author>Hoffmann</Author><Year>1990</Year><RecNum>14764</RecNum><DisplayText>[40]</DisplayText><record><rec-number>14764</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596026736">14764</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Hofmann,

				<p>hors</contributors><titles><title>Dodecyl dimethylamine oxide, CASRN: 1643-20-5, EC number: 216-700-6, Surface Tension</title><secondary-title>European Chemicals Agency</secondary-title></titles><periodical><full-title>European Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/registration-dossier/-/registered-dossier/10062/4/11</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]</p>	<p>H. </author></authors></contributors><titles><title>Correlation between surface and interfacial tensions with micellar structures and properties of surfactant solutions</title><secondary-title>Progress in Colloid & Polymer Science</secondary-title></titles><periodical><full-title>Progress in Colloid & Polymer Science</full-title></periodical><pages>16-28, https://link.springer.com/chapter/10.1007%2FBFb0116238</pages><volume>18</volume><dates><year>1990</year></dates><urls></urls></record></Cite></EndNote>]</p> <p>1×10⁻⁵ M to 5.5×10⁻⁵ M or 0.0002 to 0.001</p>
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					wt% at 25°C [ADDIN EN.CITE <EndNote><Cite><A uthor>Mukerjee</Aut hor><Year>1971</Y ear><RecNum>1476 5</RecNum><Displa yText>[41]</Display Text><record><rec- number>14765</rec- number><foreign- keys><key app="EN" db- id="sp9w2fxejsw0zre 0azr5evearxfds0err5s r" timestamp="1596026 897">14765</key></ foreign-keys><ref- type name="Journal Article">17</ref- type><contributors>< authors><author>Mu kerjee, P.</author><author> Mysels, K.J.</author></autho rs></contributors><ti tles><title>Critical micelle concentrations of aqueous surfactant systems</title><seco
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					<p>ndary-title>Prepared under contract for the Office of Standard Reference Data, National Bureau of Standards of NSRDS-NBS 36, Washington, DC 20234</secondary-title></titles><periodical><full-title>Prepared under contract for the Office of Standard Reference Data, National Bureau of Standards of NSRDS-NBS 36, Washington, DC 20234</full-title></periodical><pages>242, https://nvlpubs.nist.gov/nistpubs/Legacy/NSRDS/nbsnsrds36.pdf</pages><dates><year>1971</year></dates><urls></urls></record></Cite></EndNote>]</p>
Anionic Surfactants					
Chemical	Other Relevant Names	Criteria 1	Criteria 2	Criteria 3	

Name in Text		Hydrophobic group(s)	Hydrophilic group(s)	Surface Tension	Critical Micelle Concentration (CMC)
sodium dodecyl sulfate (SDS) CASRN: 151-21-3	CAS Name: sulfuric acid monododecyl ester sodium salt (1:1)	dodecyl group	sulfate group	35 mN/m at 0.29 wt% and 20°C [ADDIN EN.CITE <EndNote><Cite><Author>Hernainz</Author><Year>2002</Year><RecNum>14768</RecNum><DisplayText>[42]</DisplayText><record><rec-number>14768</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5eearxfds0err5sr" timestamp="1596027363">14768</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Hernainz, F.</author><author>Caro, A.</author></authors></contributors><titles><title>Variation of surface tension in	8.25×10 ⁻³ M or 0.24 wt% at 20°C [ADDIN EN.CITE <EndNote><Cite><Author>Mukerjee</Author><Year>1971</Year><RecNum>14765</RecNum><DisplayText>[41]</DisplayText><record><rec-number>14765</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5eearxfds0err5sr" timestamp="1596026897">14765</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Mukerjee, P.</author><author>Mysels, K.J.</author></authors></contributors><titles><title>Critical

				<p>aqueous solutions of sodium dodecyl sulfate in the flotation batch</title><secondary-title>Colloids and Surfaces A: Physicochemical and Engineering Aspects</secondary-title></titles><periodical><full-title>Colloids and Surfaces A: Physicochemical and Engineering Aspects</full-title></periodical><pages>19-24, https://www.sciencedirect.com/science/article/abs/pii/S0927775701005751</pages><volume>196</volume><number>1</number><dates><year>2002</year></dates><urls></urls></record></Cite></EndNote>]</p>	<p>micelle concentrations of aqueous surfactant systems</title><secondary-title>Prepared under contract for the Office of Standard Reference Data, National Bureau of Standards of NSRDS-NBS 36, Washington, DC 20234</secondary-title></titles><periodical><full-title>Prepared under contract for the Office of Standard Reference Data, National Bureau of Standards of NSRDS-NBS 36, Washington, DC 20234</full-title></periodical><pages>242, https://nvlpubs.nist.gov/nistpubs/Legacy/NSRDS/nbsnsrds36.pdf</pages><dates><year>1971</year></dates><urls></urls></record></Cite></EndNote>]</p>
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oleoyl sarcosine CASRN: 110-25-8	CAS Name: glycine, N-methyl-N-((9Z)-1-oxo-9-octadecen-1-yl)	oleyl group	carboxylic acid anion	31.91 mN/m at 0.1 wt% and 19.9°C** [ADDIN EN.CITE <EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14767</RecNum><DisplayText>[43]</DisplayText><record><rec-number>14767</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evarxfs0err5sr" timestamp="1596027202">14767</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Registration Dossier</author></authors></contributors><titles><title>Sodium N-methyl-N-(1-oxo-9-octadecenyl)aminoacetate, CASRN 3624-77-9, EC number: 222-829-9, Surface Tension</title><second	2.6×10 ⁻³ wt% and ~25°C ** (temperature not reported, assumed to be room temperature) [ADDIN EN.CITE <EndNote><Cite><Author>ChattemChemicals</Author><Year>2020</Year><RecNum>14769</RecNum><DisplayText>[44]</DisplayText><record><rec-number>14769</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evarxfs0err5sr" timestamp="1596027596">14769</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>ChattemChemicals</author></authors></contributors><titles><title>Oleoyl Sarcosine, CASRN 110-25-
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				dary-title>European Chemicals Agency</secondary- title></titles><periodic al><full- title>European Chemicals Agency</full- title></periodical><pa ges>https://www.echa. europa.eu/fi/web/guest /registration-dossier/- /registered- dossier/5350/4/11</pa ges><dates><year>20 20</year></dates><url s></urls></record></C ite></EndNote>]	8</title><secondary- title>Product Information</seconda ry- title></titles><period ical><full- title>Product Information</full- title></periodical><p ages>https://www.ch attemchemicals.com/ </pages><dates><yea r>2020</year></date s></urls></urls></rec ord></Cite></EndNo te>]
sodium lauroyl sarcosinate CASRN: 137-16-6	CAS Name: glycine, N- methyl-N-(1-oxododecyl)-, sodium salt (1:1)	lauryl group	carboxylic acid anion	40.5 mN/m at 2 wt% and 20°C [ADDIN EN.CITE <EndNote><Cite><Au thor>Dossier</Author ><Year>2020</Year> <RecNum>14770</Re cNum><DisplayText> [45]</DisplayText><r ecord><rec- number>14770</rec- number><foreign- keys><key app="EN" db- id="sp9w2fxejsw0zre0	8.0×10 ⁻² wt% and ~25°C (temperature not reported, assumed to be room temperature) [ADDIN EN.CITE <EndNote><Cite><A uthor>ChattemChemi cals</Author><Year >2020</Year><RecN um>14769</RecNum ><DisplayText>[44] </DisplayText><reco rd><rec- number>14769</rec-

				<p>azr5evealxfs0err5sr" timestamp="1596027817">14770</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Registration Dossier</author></authors></contributors><titles><title>Sodium N-lauroylsarcosinate, CASRN 137-16-6, EC number: 205-281-5, Surface Tension</title><secondary-title>European Chemicals Agency</secondary-title></titles><periodical><full-title>European Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/registration-dossier/-/registered-dossier/14123/4/11</pages><dates><year>2020</year></dates><u</p>	<p>number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evealxfs0err5sr" timestamp="1596027596">14769</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>ChattemChemicals</author></authors></contributors><titles><title>Oleoyl Sarcosine, CASRN 110-25-8</title><secondary-title>Product Information</secondary-title></titles><periodical><full-title>Product Information</full-title></periodical><pages>https://www.chattemchemicals.com/</pages><dates><year>2020</year></dates></urls></urls></rec</p>
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				rls></urls></record></Cite></EndNote>]	ord></Cite></EndNote>]
dioctyl sulfosuccinate sodium salt (DOSS) CASRN: 577-11-7	dioctyl sodium sulfosuccinate CAS Name: Butanedioic acid, 2-sulfo-, 1,4-bis(2-ethylhexyl) ester, sodium salt	two 2-ethyl hexyl groups	sulfosuccinate group	<28 mN/m at 0.5 vol% and 25°C* [ADDIN EN.CITE <EndNote><Cite><Author>Williams</Author><Year>1957</Year><RecNum>14755</RecNum><DisplayText>[46]</DisplayText><record><rec-number>14755</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evarxfs0err5sr" timestamp="1596024180">14755</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Williams, E.F.</author><author>Woodberry, N.T.</author><author>Dixon, J.K.</author></authors></contributors><titles><title>Purification	6.8×10 ⁻⁴ M or 0.03 wt% at 25°C [ADDIN EN.CITE <EndNote><Cite><Author>Mukerjee</Author><Year>1971</Year><RecNum>14765</RecNum><DisplayText>[41]</DisplayText><record><rec-number>14765</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evarxfs0err5sr" timestamp="1596026897">14765</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Mukerjee, P.</author><author>Mysels, K.J.</author></authors></contributors><titles><title>Critical micelle

				and surface tension properties of alkyl sodium sulfosuccinates</title><secondary-title>Journal of Colloid Science</secondary-title></titles><periodical><full-title>Journal of Colloid Science</full-title></periodical><pages>452-459</pages><volume>12</volume><number>5</number><dates><year>1957</year></dates><urls></urls></record></Cite></EndNote>]	concentrations of aqueous surfactant systems</title><secondary-title>Prepared under contract for the Office of Standard Reference Data, National Bureau of Standards of NSRDS-NBS 36, Washington, DC 20234</secondary-title></titles><periodical><full-title>Prepared under contract for the Office of Standard Reference Data, National Bureau of Standards of NSRDS-NBS 36, Washington, DC 20234</full-title></periodical><pages>242, https://nvlpubs.nist.gov/nistpubs/Legacy/NSRDS/nbsnsrds36.pdf </pages><dates><year>1971</year></dates><urls></urls></record></Cite></EndNote>]
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Cationic Surfactants					
Chemical Name in Text	Other Relevant Names	Criteria 1		Criteria 2	Criteria 3
		Hydrophobic group(s)	Hydrophilic group(s)	Surface Tension	Critical Micelle Concentration (CMC)
benzalkonium chloride (BAC) CASRN: 8001-54-5	CAS Name: quaternary ammonium compounds, alkylbenzyl dimethyl, chlorides	alkyl chains are C12, C14, C16 and C18 and benzyl group	quaternary nitrogen	37 mN/m at concentrations greater than about 4×10^{-4} M and 25°C* [ADDIN EN.CITE <EndNote><Cite><Author>Nandni</Author><Year>2013</Year><RecNum>14766</RecNum><DisplayText>[47]</DisplayText><record><rec-number>14766</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evealxfs0err5sr" timestamp="1596027033">14766</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Nandni,	C12: reported values range from 2.3 - 8.5×10^{-3} M or 0.078 - 0.29 wt% at 25°C C14: 3.7×10^{-4} M or 0.014 wt% and ~25°C (temperature not stated; assumed to be room temperature) C16: 4.2×10^{-5} M or 0.0016 wt% at 23°C C18: reported values range from 7.1 - 8.5×10^{-6} M or 0.0003 - 0.00036 wt% at 23°C [ADDIN EN.CITE <EndNote><Cite><Author>Mukerjee</Author><Year>1971</Year><RecNum>14765</RecNum><DisplayText>[41]</Display

				D.</author><author> Mahajan, R.K.</author></author s></contributors><title s><title>Micellar and Interfacial Behavior of Cationic Benzalkonium Chloride and Nonionic Polyoxyethylene Alkyl Ether Based Mixed Surfactant Systems</title><secon dary-title>Journal of Surfactants and Detergents</secondary - title></titles><periodic al><full-title>Journal of Surfactants and Detergents</full- title></periodical><pa ges>587-599, https://doi.org/10.1007 /s11743-012-1427- z</pages><volume>16 </volume><number>4 </number><dates><ye ar>2013</year></date s><urls></urls></reco rd></Cite></EndNote >]	Text><record><rec- number>14765</rec- number><foreign- keys><key app="EN" db- id="sp9w2fxejsw0zre 0azr5evealxfds0err5s r" timestamp="1596026 897">14765</key></ foreign-keys><ref- type name="Journal Article">17</ref- type><contributors>< authors><author>Mu kerjee, P.</author><author> Mysels, K.J.</author></autho rs></contributors><ti tles><title>Critical micelle concentrations of aqueous surfactant systems</title><seco ndary-title>Prepared under contract for the Office of Standard Reference Data, National Bureau of Standards of NSRDS- NBS 36, Washington, DC
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					20234</secondary-title></titles><periodical><full-title>Prepared under contract for the Office of Standard Reference Data, National Bureau of Standards of NSRDS-NBS 36, Washington, DC 20234</full-title></periodical><pages>242, https://nvlpubs.nist.gov/nistpubs/Legacy/NSRDS/nbsnsrds36.pdf </pages><dates><year>1971</year></dates><urls></urls></record></Cite></EndNote>]
didecyl dimethyl ammonium chloride (DDAC) CASRN: 7173-51-5	CAS Name: 1-decanaminium, N-decyl-N,N-dimethyl-, chloride (1:1)	decyl groups	quaternary nitrogen	25.82 mN/m at 1 g/L (0.1 wt%) and 20°C [ADDIN EN.CITE <EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14771</RecNum><DisplayText>[48]</DisplayText><record><rec-number>14771</rec-number><foreign-	0.39 g/L or 0.039 wt% at 25°C [ADDIN EN.CITE <EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14771</RecNum><DisplayText>[48]</DisplayText><record><rec-number>14771</rec-number><foreign-

				keys><key app="EN" db- id="sp9w2fxejsw0zre0 azr5evealrxfds0err5sr" timestamp="15960279 46">14771</key></for eign-keys><ref-type name="Journal Article">17</ref- type><contributors><a uthors><author>Regist ration Dossier</author></aut hors></contributors>< titles><title>Didecylidi methylammonium chloride, CASRN: 7173-51-5, EC number: 230-525-2, Surface Tension</title><secon dary-title>European Chemicals Agency</secondary- title></titles><periodic al><full- title>European Chemicals Agency</full- title></periodical><pa ges>https://echa.europ a.eu/registration- dossier/-/registered-	keys><key app="EN" db- id="sp9w2fxejsw0zre 0azr5evealrxfds0err5s r" timestamp="1596027 946">14771</key></ foreign-keys><ref- type name="Journal Article">17</ref- type><contributors>< authors><author>Reg istration Dossier</author></au thors></contributors> <titles><title>Didecy ldimethylammonium chloride, CASRN: 7173-51-5, EC number: 230-525-2, Surface Tension</title><seco ndary-title>European Chemicals Agency</secondary- title></titles><period ical><full- title>European Chemicals Agency</full- title></periodical><p ages>https://echa.eur opa.eu/registration-
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				dossier/5864/4/11</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]	dossier/-/registered-dossier/5864/4/11</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]
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*Not all of the surface tension measurement references identified are run at exactly 20°C, but they are sufficiently close (within 5°C) so as not to affect the measurement. In addition, several measurements were run at 0.1% instead of the recommended 0.5%. Increasing the concentration to 0.5% is likely to lower the surface tension.

**Carboxylic acid compounds, such as oleoyl sarcosine, have a carboxyl group pKa value of ~5, thus at physiological pH values maintained near 7 in the lung, the carboxyl group will be 99% in the anionic form according the Henderson-Hasselbalch equation. Since sodium is the major cation in mammalian body fluids (~145 mM), the use of the sodium oleoyl sarcosine surface tension value is appropriate for its characterization.

***Amphoteric: At pH 7, 90% expected to be nonionic; only small amount cationic.

Hazard Identification

There is concern for dysfunction of mucus, epithelial lining fluid, and natural surfactant lining in the various regions of the respiratory tract from inhalation of surfactants. There is also evidence that some surfactants or similar structures may also interfere with the cell membrane of the epithelium in these same regions [ADDIN EN.CITE ADDIN EN.CITE.DATA]. This effect on cell membranes is apparent from data on numerous surfactants indicating irritation to the skin and eye, as noted below. The capacity of exogenous surfactants to interfere with pulmonary surfactant and impair pulmonary function has been demonstrated in both human volunteers and in laboratory animals [51, 5-7]. The respiratory tract responses to inhaled surfactant aerosol is thought to be in proportion to the exposure concentration and duration, but available data on acute and repeated-dose effect levels are limited within each subcategory, which limits establishing a correlation between chemical properties and toxicity due to exposure methods (*e.g.*, generated aerosol droplet size).

Nonionic Surfactants

In vivo studies

Several studies were identified for the nonionic siliconized superinone respiratory detergent, 4-(1,1,3,3-tetramethylbutyl)phenol polymer with formaldehyde and oxirane (CASRN 25301-02-4; commonly known as Defomarie, Alevaire, and Tyloxapol). Healthy human volunteers demonstrated significantly decreased respiratory compliance following acute inhalation of Defomarie [ADDIN EN.CITE

<EndNote><Cite><Author>Obenour</Author><Year>1963</Year><RecNum>13656</RecNu

m><DisplayText>[51]</DisplayText><record><rec-number>13656</rec-number><foreign-
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A.</author><author>Saltzman, H. A.</author><author>Sieker, H. O.</author><author>Green,
J. L.</author></authors></contributors><titles><title>Effects of surface-active aerosols and
pulmonary congestion on lung compliance and resistance</title><secondary-
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title>Circulation</full-title><abbr-1>Circulation</abbr-1></periodical><alt-periodical><full-
title>Circulation</full-title><abbr-1>Circulation</abbr-1></alt-periodical><pages>888-
92</pages><volume>28</volume><edition>OBENOUR, R ASALTZMAN, H
ASIEKER, H OGREEN, J
L1963/11/01</edition><keywords><keyword>Aerosols</keyword><keyword>Alcohols
</keyword><keyword>Ethanol</keyword><keyword>Heart
Failure</keyword><keyword>Humans</keyword><keyword>Infusions,
Parenteral</keyword><keyword>Injections,
Intravenous</keyword><keyword>Lung</keyword><keyword>Lung
Compliance</keyword><keyword>Pulmonary Edema</keyword><keyword>Respiratory
Function Tests</keyword><keyword>Silicones</keyword><keyword>Sodium
Chloride</keyword><keyword>Surface-Active
Agents</keyword></keywords><dates><year>1963</year><pub-
dates><date>Nov</date></pub-dates></dates><isbn>0009-7322 (Print)0009-7322
(Linking)</isbn><accession-num>14079193</accession-num><call-num>0 (Aerosols)0

(Alcohols)0 (Silicones)0 (Surface-Active Agents)3K9958V90M
(Ethanol)451W47IQ8X (Sodium Chloride)</call-num><urls></urls><remote-database-
provider>NLM</remote-database-
provider><language>Eng</language></record></Cite></EndNote>]. An increased minimum
surface tension due to detergent was shown to be dose-dependent, using pulmonary surfactant
extracted from dogs with the nonionic surfactant tyloxapol (Alevaire) *in vitro* [ADDIN
EN.CITE ADDIN EN.CITE.DATA]. However, *in vivo* exposure of dogs to Alevaire (8-
hour aerosol exposure; vehicle, particle size and distribution, and concentration not reported)
produced little effect (only 1/10 dogs exposed to Alevaire showed increased minimum surface
tension). The results did not support the dose-dependence of the effect and indicated that small
amounts of detergent in the lungs may not detectably alter the surface tension-surface area
relationship and that alteration of surface tension is unlikely to occur during reasonable use
although there is considerable uncertainty regarding the internal dose achieved [ADDIN
EN.CITE ADDIN EN.CITE.DATA].

Inhalation studies using dogs and/or sheep exposed to nonionic surfactant, tyloxapol, resulted in
reduced oxygen content of arterial blood due to impaired gas exchange in the lung, increased
pulmonary extravascular water volume and wet-to-dry weight ratio of the lungs, and grossly
visible pulmonary edema and atelectasis (*i.e.*, collapsed alveoli) [ADDIN EN.CITE ADDIN
EN.CITE.DATA]. In the study by Modell *et al.* (1969) [ADDIN EN.CITE ADDIN
EN.CITE.DATA], no gross pathology differences were seen in detergent-exposed versus
control lungs of dogs, although some portions of both control and exposed lungs were heavy and
discolored reddish-purple, which may have been caused by fluid accumulation from the liquid

aerosol exposures and/or the use of hypotonic saline in the study (0.45% NaCl) since these effects were not observed in lungs treated with a less dense aerosol. Normal appearances were observed in the remaining areas of the lungs.

In rodents, irritation and inflammatory effects in the entire respiratory tract have been observed with varying degrees of severity. Acute inhalation exposure *via* nose-only administration for 4 hours in Wistar Han rats to a concentration of 5.1 mg/L (5,100 mg/m³) with an MMAD of 2.2 µm and a GSD of 2 to Sorbitan monolaurate, ethoxylated (CASRN 9005-64-5), a chemical not irritating to the skin or eyes [ADDIN EN.CITE

<EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14776</RecNum><DisplayText>[52]</DisplayText><record><rec-number>14776</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evealxfs0err5sr" timestamp="1596030693">14776</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Registration Dossier</author></authors></contributors><titles><title>Sorbitan monolaurate, ethoxylated, 1 - 6.5 moles ethoxylated, CASRN: 9005-64-5, EC number: 500-018-3, Skin irritation/corrosion</title><secondary-title>European Chemicals Agency</secondary-title></titles><periodical><full-title>European Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/hr/registration-dossier/-/registered-dossier/13525/7/4/2</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>], did not result in an increase in mortalities, clinical signs, or abnormalities in the gross pathology [ADDIN EN.CITE

<EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14777</RecNum>

<DisplayText>[53]</DisplayText><record><rec-number>14777</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evealrfs0err5sr" timestamp="1596030813">14777</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Registration Dossier</author></authors></contributors><titles><title>Sorbitan monolaurate, ethoxylated 1 - 6.5 moles ethoxylated, CASRN: 9005-64-5, EC number: 500-018-3, Acute Toxicity: Inhalation</title><secondary-title>European Chemicals Agency</secondary-title></titles><periodical><full-title>European Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/hr/registration-dossier/-/registered-dossier/13525/7/3/3</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]. A respiratory irritation study using plethysmography was performed on a mixture containing octylphenoxypolyethoxyethanol [ADDIN EN.CITE ADDIN EN.CITE.DATA], which can be severely irritating to the skin and eyes, in male Webster mice exposed for 3 hours to concentrations of 12, 22, 51, 118, and 134 mg/m³ with 30-60 minutes recovery time (MMAD and GSD not provided). Signs of pulmonary irritation were observed in animals at the two highest concentrations as indicated by a decrease in respiratory frequency (33-58% decrease); this response was preceded by an increase in respiratory frequency (11-12.5% increase) at the highest three concentrations without an increase in gross lung abnormalities, pulmonary edema, or lung weight [ADDIN EN.CITE <EndNote><Cite><Author>Alarie</Author><Year>1992</Year><RecNum>14778</RecNum><DisplayText>[54]</DisplayText><record><rec-number>14778</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evealrfs0err5sr" timestamp="1596035219">14778</key></foreign-keys><ref-type name="Journal

Article">17</ref-type><contributors><authors><author>Alarie, Y.</author><author>Stock, M.F.</author></authors></contributors><titles><title>Respiratory Irritancy on a Mixture containing Polyethylene Glycol Mono(Octyl)Phenyl Ether CAS #9035-19-5</title><secondary-title>ChemView - U.S. Environmental Protection Agency</secondary-title></titles><periodical><full-title>ChemView - U.S. Environmental Protection Agency</full-title></periodical><pages>37, https://chemview.epa.gov/chemview/proxy?filename=09022526800b76c9_86960000465_09-26-2011_8D_PHCS_Original%20-%2086960000465.pdf</pages><dates><year>1992</year></dates><urls></urls></record></Cite></EndNote>]. An acute inhalation exposure study in Syrian hamsters exposed to 3.0 mg/L of octylphenoxypolyethoxyethanol with varying exposure durations showed that lung deposition directly corresponded to mortality with an LD₅₀ of 1300-2100 µg with an MMAD of 1.47 µm and a GSD of 1.84 [ADDIN EN.CITE <EndNote><Cite><Author>Damon</Author><Year>1982</Year><RecNum>13323</RecNum><DisplayText>[55]</DisplayText><record><rec-number>13323</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evealxfs0err5sr" timestamp="1479320592">13323</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Damon, E. G.</author><author>Halliwell, W. H.</author><author>Henderson, T. R.</author><author>Mokler, B. V.</author><author>Jones, R. K.</author></authors></contributors><titles><title>Acute toxicity of polyethylene glycol p-isooctylphenol ether in syrian hamsters exposed by inhalation or bronchopulmonary lavage</title><secondary-title>Toxicology and applied pharmacology</secondary-title><alt-

title>Toxicol Appl Pharmacol</alt-title></titles><periodical><full-title>Toxicology and Applied
 Pharmacology</full-title><abbr-1>Toxicol. Appl. Pharmacol.</abbr-1></periodical><pages>53-
 61</pages><volume>63</volume><number>1</number><edition>Damon, E
 GHalliwell, W HHenderson, T RMokler, B VJones, R
 K1982/03/30</edition><keywords><keyword>Animals</keyword><keyword>Cricetinae
 </keyword><keyword>Detergents/ toxicity</keyword><keyword>Dose-Response Relationship,
 Drug</keyword><keyword>Female</keyword><keyword>Lethal Dose
 50</keyword><keyword>Lung/ drug
 effects/pathology</keyword><keyword>Male</keyword><keyword>Mesocricetus</keyword><
 keyword>Octoxynol</keyword><keyword>Polyethylene Glycols/administration & dosage/
 toxicity</keyword><keyword>Surface-Active Agents/
 toxicity</keyword><keyword>Therapeutic
 Irrigation</keyword></keywords><dates><year>1982</year><pub-dates><date>Mar
 30</date></pub-dates></dates><isbn>0041-008X (Print)0041-008X
 (Linking)</isbn><accession-num>7071873</accession-num><call-num>0 (Detergents)0
 (Surface-Active Agents)30IQX730WE (Polyethylene Glycols)9002-93-1
 (Octoxynol)</call-num><urls></urls><remote-database-provider>NLM</remote-database-
 provider><language>Eng</language></record></Cite></EndNote>]. The deaths in these
 animals were attributed to severe laryngeal edema and ulcerative laryngitis while the lower
 airways in these animals were relatively free of serious pathologies which likely indicates limited
 deposition to the lower airways in this study. The authors hypothesized that these observed
 effects were due to large tracheobronchial deposition following the aerosol exposure and the
 mucociliary clearance of the chemical resulted in a large concentration on the laryngeal mucosa,

though laryngeal deposition is typically a function of aerodynamics. In the only 2-week whole-body inhalation study for nonionic surfactants, male and female Sprague-Dawley rats were exposed to 5.3 and 10.3 mg/m³ (5/sex/dose; MMAD 1.8 µm, GSD 1.8) octylphenoxypolyethoxyethanol for 6 hours/day, 5 days/week [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Slight to minimal subacute inflammation of the alveolar walls and hyperplasia of the alveolar/bronchiolar epithelium was reported, in addition to an increase in slight discoloration of the lungs, increased lung weight, and mucoid nasal discharge; a LOAEC of 5.3 mg/m³ was identified.

Mechanistic studies

In vitro studies of surfactant on cell membranes have provided evidence of possible modes of action (MOAs). Warisnoicharoen *et al.* (2003) [ADDIN EN.CITE ADDIN EN.CITE.DATA] evaluated the cytotoxicity of the nonionic surfactants polyoxyethylene-10-oleyl ether (C_{18:1}E₁₀; CASRN 9004-98-2), polyoxyethylene-10-dodecyl ether (C₁₂E₁₀; CASRN 9002-92-0), and N,N-dimethyl-dodecylamine-N-oxide (C₁₂AO; CASRN 1643-20-5) on submerged cultured human bronchial epithelium cells (16-HBE14o-) *in vitro*, using the MTT cell viability assay by exposing the cells to 0.1mL of the serially diluted microemulsion (particle size not reported) for 30 minutes followed by a 60 minute incubation with a MTT solution. All surfactants tested were cytotoxic at concentrations near or below their critical aggregation (micellular) concentrations (as determined by surface tension measurements), suggesting that toxicity was due to the disruption caused by the partitioning of monomeric surfactant into the cell membrane.

Lindenberg *et al.* (2019) [ADDIN EN.CITE

<EndNote><Cite><Author>Lindenberg</Author><Year>2019</Year><RecNum>14779</Rec

Num><DisplayText>[57]</DisplayText><record><rec-number>14779</rec-number><foreign-

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Article">17</ref-type><contributors><authors><author>Lindenberg,

F.</author><author>Sichel, F.</author><author>Lechevrel, M.</author><author>Respaud,

R.</author><author>Saint-Lorant,

G.</author></authors></contributors><titles><title>Evaluation of Lung Cell Toxicity of

Surfactants for Inhalation Route</title><secondary-title>Journal of Toxicology and risk

assessment</secondary-title></titles><periodical><full-title>Journal of Toxicology and risk

assessment</full-title></periodical><pages>https://doi.org/10.23937/2572-

4061.1510022</pages><volume>5</volume><number>1</number><dates><year>2019</year>

</dates><urls></urls></record></Cite></EndNote>] evaluated the cytotoxic activity of the three

nonionic polymeric surfactants Polysorbate 20 (CASRN 9005-64-5), Polysorbate 80 (Tween 80)

and Poloxamer 188 (CASRN 691397-13-4), which are commonly used in formulations of

Commented [A26]: Space inserted

nebulized pharmaceuticals to prevent protein agglomeration, in a BEAS-2B human bronchial

epithelial cell model using an innovative air-liquid interface (ALI) method of exposure with a

nasal spray system (MMAD and GSD not provided). In this study, the ALI results were

compared to the classical submerged cell culture or liquid/liquid (L/L) model. The study

measured the release of lactate dehydrogenase (LDH), an intercellular enzyme present in the

cytoplasm, indicative of the loss of membrane integrity. Cytotoxicity of Polysorbate 20 was

observed at concentrations of 1-2% (v/v) when using the more biologically relevant ALI method;

however, a significant increase in LDH was only observed at 4% for Polysorbate 80 and not significantly increased at concentrations of up to 10% for Poloxamer 188. These results suggest that Polysorbate 20 and to a lesser extent, Polysorbate 80 induce damage to the cell membrane integrity while the linear Poloxamer 188 did not demonstrate any *in vitro* cytotoxicity.

The available *in vitro* and *in vivo* data indicate inconsistency in respiratory toxicity among nonionic surfactants; however, the degree to which the variation is due to experimental design or bioactivity of the surfactant is not discernible from these data. The small dataset presented in this section preclude establishing correlations between respiratory effects and chemical properties, such as surface tension or CMC. Similarly, the examination of the relationship between chemical properties of nonionic surfactants and eye irritation has not established that hydrophilic-

lipophilic balance, pH, alkyl chain length, or poly [oxyethylene] chain lengths can be used to predict eye irritation potential across the nonionic surfactant subcategory [ADDIN EN.CITE

<EndNote><Cite><Author>Heinze</Author><Year>1999</Year><RecNum>14780</RecNum

><DisplayText>[58]</DisplayText><record><rec-number>14780</rec-number><foreign-

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Article">17</ref-type><contributors><authors><author>Heinze,

J.E.</author><author>Casterton, P.L.</author><author>Atrash,

J.</author></authors></contributors><titles><title>Relative Eye Irritation Potential of Nonionic

Surfactants: Correlation to Dynamic Surface Tension</title><secondary-title>Journal of

toxicology: cutaneous and ocular toxicology</secondary-title></titles><periodical><full-

title>Journal of toxicology: cutaneous and ocular toxicology</full-

title></periodical><pages>359-374,

https://doi.org/10.3109/15569529909065552</pages><volume>18</volume><dates><year>199

9</year></dates><urls></urls></record></Cite></EndNote>]. However, significant correlations

of eye irritation and the maximum reduction in surface tension were observed at the CMC or

higher surfactant concentration when surface tension was measured under dynamic conditions

(0.24, 1, and 4 bubbles/second). Whether this chemical property similarly predicts potency of

nonionic surfactants for respiratory effects requires additional data and analysis outside of the

scope of this summary.

Anionic Surfactants

In vivo studies

Two acute inhalation toxicity studies were identified for anionic surfactants, both demonstrated

high toxicity *via* the inhalation route. Oleoyl sarcosine (CASRN 110-25-8), irritating to the skin

and damaging to the eye [ADDIN EN.CITE

<EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14781</RecNum

><DisplayText>[59]</DisplayText><record><rec-number>14781</rec-number><foreign-

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Article">17</ref-type><contributors><authors><author>Registration

Dossier</author></authors></contributors><titles><title>N-methyl-N-[C18-

(unsaturated)alkanoyl]glycine, CASRN: NA, EC number: 701-177-3, Skin

irritation/corrosion</title><secondary-title>European Chemicals Agency</secondary-

title></titles><periodical><full-title>European Chemicals Agency</full-
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 dossier/21429/7/4/2/?documentUUID=fbaef057-ecc7-4763-aa56-
 1fa2c88c606c</pages><dates><year>2020</year></dates><urls></urls></record></Cite></End
 Note>], was evaluated in a 4-hour nose-only inhalation study in male and female Sprague-
 Dawley rats at concentrations of 0.3, 0.6, 2.2, and 3.7 mg/L (300, 600, 2,200, 3,700 mg/m³). The
 MMAD and GSD were not reported. An LC₅₀ of 1.37 mg/L was identified with edema of the
 lung at 0.6 mg/L and audible gasping at 0.3 mg/L. For sodium lauroyl sarcosinate (CASRN 137-
 16-6), irritating to the skin and corrosive to the eye (undiluted) [ADDIN EN.CITE
 <EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14782</RecNum>
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 CASRN: 137-16-6, EC number: 205-281-5, Eye Irritation</title><secondary-title>European
 Chemicals Agency</secondary-title></titles><periodical><full-title>European Chemicals
 Agency</full-title></periodical><pages>https://echa.europa.eu/hr/registration-dossier/-
 /registered-
 dossier/14123/7/4/3</pages><dates><year>2020</year></dates><urls></urls></record></Cite>
 </EndNote>], 5 male Wistar rats were exposed to a 4-hour nose-only inhalation concentration of
 0.05, 0.5, 1, and 5 mg/L (50, 500, 1,000, and 5,000 mg/m³) with a MMAD of 4.4, 2.9, 3.7, and
 6.0 µm; and GSD of 2.7, 3, 4.2, and 2.9, respectively. Additionally, 5 female rats were exposed

to 1.1 or 5.5 mg/L with a MMAD 3.7 or 6.0 µm and GSD of 4.2 or 2.9, respectively [ADDIN EN.CITE

<EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14782</RecNum>
><DisplayText>[60, 61]</DisplayText><record><rec-number>14782</rec-number><foreign-
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Dossier</author></authors></contributors><titles><title>Sodium N-lauroylsarcosinate,
CASRN: 137-16-6, EC number: 205-281-5, Eye Irritation</title><secondary-title>European
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dossier/14123/7/4/3</pages><dates><year>2020</year></dates><urls></urls></record></Cite>
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Dossier</author></authors></contributors><titles><title>Sodium N-lauroylsarcosinate,
CASRN: 137-16-6, EC number: 205-281-5, Acute Toxicity: Inhalation</title><secondary-
title>European Chemicals Agency</secondary-title></titles><periodical><full-title>European
Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/hr/registration-
dossier/-/registered-

dossier/14123/7/3/3</pages><dates><year>2020</year></dates><urls></urls></record></Cite>
</EndNote>]. The 5 mg/L dose resulted in fatality in all 10 animals (males and females) tested
within 1-2 h of dosing and the 0.5 mg/L dose resulted in fatality for 4/5 of the males and
exposure to 1 mg/L resulted in fatalities for the 10 animals (males and females) within 1-2 days
of exposure. Males exposed to 0.05 mg/L did not demonstrate any adverse clinical signs or
mortality at the conclusion of the study. At necropsy, red foci were noted on the lungs in males
and females receiving concentrations of ≥ 0.5 mg/L. The LC_{50} was reported to be 0.05-0.5 mg/L.

Repeated-dose inhalation studies were identified for oleoyl sarcosine, and dioctyl sodium
sulfosuccinate (CASRN 577-11-7). Oleoyl sarcosine was evaluated in a 28-day nose-only
inhalation study (6 hours/day, 5 days/week; Organization for Economic Cooperation and
Development [OECD] Test Guideline [TG] 412) in male and female Fischer rats (5/group/sex)
using concentrations of 0, 0.006, 0.02, or 0.06 mg/L (0, 6, 20, or 60 mg/m³). The particle
exposure MMAD was 1.11, 1.15, or 1.22 μ m, GSD 1.68-2.57, and density 0.79 g/cm³ for 6
hours/day, 5 days/week in 10% ethanol [ADDIN EN.CITE

<EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14784</RecNum>
><DisplayText>[62]</DisplayText><record><rec-number>14784</rec-number><foreign-
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timestamp="1596036869">14784</key></foreign-keys><ref-type name="Journal
Article">17</ref-type><contributors><authors><author>Registration
Dossier</author></authors></contributors><titles><title>N-methyl-N-[C18-
(unsaturated)alkanoyl]glycine, CASRN: NA, EC number: 701-177-3, Repeated dose toxicity:
Inhalation</title><secondary-title>European Chemicals Agency</secondary-

title></titles><periodical><full-title>European Chemicals Agency</full-
title></periodical><pages>https://echa.europa.eu/hr/registration-dossier/-/registered-
dossier/21429/7/6/3</pages><dates><year>2020</year></dates><urls></urls></record></Cite>
</EndNote>]. Changes in the mean corpuscular volume (MCV), white blood cells (WBC), and
lymphocytes were observed in male animals at the high exposure concentration. In female
animals of the mid-concentration exposure group, reticulocyte counts were significantly reduced.
Reflex bradypnea was noted in the animals at the mid and high concentrations, which is
associated with severely irritating substances. All test concentrations caused effects at several
sites of the respiratory tract with indications for local irritation, such as squamous metaplasia and
epithelium proliferation and submucous acute inflammation at the base of the epiglottis. In the
alveoli walls and bronchi, the most prominent finding was a focal early stage of fibrosis, but
details were not provided at the dose level for this effect. Lung weights were increased at the
highest dose. The LOAEC was 0.006 mg/L (6 mg/m³) air in males and females; the basis for the
effect level was local irritation.

Diocetyl sulfosuccinate sodium salt (DOSS; CASRN 577-11-7) was evaluated in a 13-week
inhalation study in male and female Sprague-Dawley rats (12/group/sex). Rats were exposed to
an aerosol of a product containing 0.0042 mg/L (4.2 mg/m³) DOSS, for 4 hours a day, 5 days a
week (as reported in a secondary source; exposure details, MMAD, and GSD not reported) [

ADDIN EN.CITE

<EndNote><Cite><Author>CIR</Author><Year>2013</Year><RecNum>14785</RecNum><
DisplayText>[63]</DisplayText><record><rec-number>14785</rec-number><foreign-
keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evealrxfds0err5sr"

timestamp="1596037107">14785</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>CIR</author></authors></contributors><titles><title>Safety Assessment of Alkyl Sulfosuccinate Salts as Used in Cosmetics, Re-Review, CIR Expert Panel Meeting, June 10-11, 2013</title><secondary-title>Cosmetic Ingredient Review (CIR), Washington, D.C.</secondary-title></titles><periodical><full-title>Cosmetic Ingredient Review (CIR), Washington, D.C.</full-title></periodical><pages>171, https://www.cir-safety.org/sites/default/files/Sulfosuccinates_RR.pdf</pages><dates><year>2013</year></dates></urls></urls></record></Cite></EndNote>]. There were no statistically significant differences in exposed and control groups for the mean body weight gain, survival, appearance and behavior, urinalysis values, and microscopic lesions. Significant differences were noted in the blood as indicated by elevated erythrocytic values (not otherwise specified) at 7 weeks and depressed mean corpuscular hemoglobin concentration values at 13 weeks in male rats. In females, depressed serum glutamic pyruvic transaminase and significant effect on absolute heart weight was observed at 7 weeks, depressed serum alkaline phosphatase was observed at 13 weeks and elevated glucose at 7 and 13-weeks. At 7 weeks, the lungs of necropsied animals showed scattered foci of neutrophils and an increase in alveolar macrophages were reported in a single exposed male rat. A LOAEC of 4.2 mg/m³ was identified based on the blood effects in male rats.

Mechanistic studies

Mechanistic studies on the pulmonary effects of anionic surfactants have been studied in dogs, rabbits, and sheep exposed to DOSS.

Increased minimum surface tension of lung extract or bronchioalveolar lavage fluid (BALF) was observed in dogs and sheep following *in vivo* aerosol exposure to DOSS in 1:1 mixture of ethanol and saline for 30 – 60 minutes, at a concentration that was selected to ensure a moderate degree of edema (estimated dose of 15 mg detergent/kg body weight) [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Anesthetized dogs were exposed *via* a ventilator to particle sizes of 0.5 to 15 μm with an MMAD of 3 μm (no GSD reported). Light microscopic examination of the lungs 4 hours after exposure to DOSS aerosol observed no grossly destructive effects on alveolar cells or lung architecture in exposed dogs. However, a decrease in pulmonary compliance was observed that the authors hypothesized was due to an increase in surface tension in the alveoli in the presence of detergent.

Alveolar-capillary barrier permeability studies using radiolabeled aerosol tracers have evaluated whether detergents effect the surfactant layer to increase alveolar permeability. Inhalation exposure to DOSS enhanced the pulmonary elimination of radiolabeled diethylenetriamine pentaacetic acid (DTPA; CASRN 67-43-6) a relatively small hydrophilic molecule, indicating an increased alveolar permeability after detergent exposure [ADDIN EN.CITE ADDIN EN.CITE.DATA]. In most studies, this effect on alveolar permeability was seen in the absence of effects on blood gas levels or pulmonary compliance that occurs with higher exposure, indicating that the increase in alveolar permeability is a sensitive effect of detergent aerosol. The effect was demonstrated to be concentration-related in rabbits exposed to multiple dilutions (0.125, 0.25, 0.5, and 2%) with a MMAD of 1.7 μm of the liquid detergent [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Studies also evaluated the elimination of a radiolabeled aerosol of albumin, a much larger molecule, which was enhanced by DOSS as well, but to a lesser

degree than DTPA [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Wang *et al.* (1993) [ADDIN EN.CITE ADDIN EN.CITE.DATA] observed an increase in protein flux from plasma to alveolar space after DOSS inhalation in sheep, which was attributed to disruption of the alveolar lining and increased microvascular permeability. The increased alveolar permeability observed in these studies was hypothesized to be a result of increased alveolar surface tension, which may result in increased permeability by opening previously closed pores (through which solutes pass) in the membrane or by stretching already open pores [ADDIN EN.CITE ADDIN EN.CITE.DATA]. However, as noted, surfactants can disrupt cell membranes; thus, this mechanism may be an alternate explanation [ADDIN EN.CITE

<EndNote><Cite><Author>Burden</Author><Year>2012</Year><RecNum>14727</RecNum><DisplayText>[1]</DisplayText><record><rec-number>14727</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evealrfs0err5sr" timestamp="1596017177">14727</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Burden, D.W.</author></authors></contributors><titles><title>Guide to the Disruption of Biological Samples - 2012, Version 1.1.</title><secondary-title>Random Primers</secondary-title></titles><periodical><full-title>Random Primers</full-title></periodical><pages>1-25</pages><number>12</number><dates><year>2012</year></dates><urls></urls></record></Cite></EndNote>].

Cationic Surfactants

In vivo studies

Three acute inhalation toxicity studies were identified for cationic surfactants; one study each for

Commented [A27]: ADD LARSEN ET AL - KEITH

DDAC, dioctadecyldimethylammonium chloride (DODMAC; CASRN 107-64-2), and BAC.

DDAC, which is corrosive to the skin and severely damaging to the eye [ADDIN EN.CITE

<EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14786</RecNum>

><DisplayText>[71]</DisplayText><record><rec-number>14786</rec-number><foreign-

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Dossier</author></authors></contributors><titles><title>Didecyldimethylammonium chloride,

CASRN: 7173-51-5, EC number: 230-525-2, Skin irritation/corrosion</title><secondary-

title>European Chemicals Agency</secondary-title></titles><periodical><full-title>European

Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/hr/registration-

dossier/-/registered-

dossier/5864/7/4/2</pages><dates><year>2020</year></dates><urls></urls></record></Cite><

/EndNote>], was tested in rats (5/sex/dose, unspecified strain) exposed *via* inhalation to 0.05,

0.09, 0.13, 0.25, 1.36, or 4.54 mg/L (50, 90, 130, 250, 1,360, or 4,540 mg/m³) for 2 hours with

an observation period of 14 days (no additional exposure conditions reported). An LC₅₀ of 0.07

mg/L was identified based on unspecified abnormalities identified in several organs including the

lungs [ADDIN EN.CITE

<EndNote><Cite><Author>EPA</Author><Year>2006</Year><RecNum>14845</RecNum><

DisplayText>[72]</DisplayText><record><rec-number>14845</rec-number><foreign-

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timestamp="1597755265">14845</key></foreign-keys><ref-type name="Journal

Article">17</ref-

type><contributors><authors><author>EPA</author></authors></contributors><titles><title>R
registration Eligibility Decision for Aliphatic Alkyl Quaternaries (DDAC)</title><secondary-
title>Office of Chemical Safety and Pollution Prevention, Office of Pesticide Programs, U.S.
Environmental Protection Agency, Washington, D.C. 20460</secondary-
title></titles><periodical><full-title>Office of Chemical Safety and Pollution Prevention, Office
of Pesticide Programs, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-
title></periodical><pages>127,
https://archive.epa.gov/pesticides/reregistration/web/pdf/ddac_red.pdf</pages><volume>EPA73
9-R-06-

008</volume><dates><year>2006</year></dates><urls></urls></record></Cite></EndNote>].

A similar quaternary amine, DODMAC, which is irritating to the skin and causes serious damage
to the eyes [ADDIN EN.CITE

<EndNote><Cite><Author>EURAR</Author><Year>2009</Year><RecNum>14787</RecNu
m><DisplayText>[73]</DisplayText><record><rec-number>14787</rec-number><foreign-
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e>European Union Risk Assessment Report (EURAR), CAS No: 107-64-2, EINECS No: 203-
508-2, dimethyldioctadecylammonium chloride (DODMAC)</title><secondary-title>European
Commission, Joint Research Centre, Institute for Health and Consumer Protection (IHCP),
former Toxicology and Chemical Substances (TCS) European Chemicals Bureau

(ECB)</secondary-title></titles><periodical><full-title>European Commission, Joint Research Centre, Institute for Health and Consumer Protection (IHCP), former Toxicology and Chemical Substances (TCS) European Chemicals Bureau (ECB)</full-title></periodical><pages>123, <https://echa.europa.eu/documents/10162/46f2f114-12ff-4af4-8da7-72148b6a202e></pages><volume>14</volume><dates><year>2009</year></dates><urls></urls></record></Cite></EndNote>], was tested in albino rats (10 males, strain not specified) to the test substance (1:29 distilled water) *via* inhalation at 180 mg/L (180,000 mg/m³) for one hour and observed for 14 days (no additional exposure conditions reported). No mortalities were reported and observed treatment-related clinical signs included preening, excessive masticatory (chewing) movements, excessive salivation stains, lacrimation, serosanguineous stains around the nose, and labored respiration. All animals appeared normal one day after dosing. The LC₅₀ (1 h) was > 180 mg/L. BAC, which is corrosive to the skin and causes severe eye damage [ADDIN EN.CITE ADDIN EN.CITE.DATA], was tested in female Wistar rats (5/group) exposed *via* nose-only inhalation to 37.6 and 53 mg/m³ for 4 hours and observed for 14 days or exposed to 30.6 mg/m³ for 6 hours and BALF was measured 18 hours post-exposure (MMAD and GSD not reported) [ADDIN EN.CITE <EndNote><Cite><Author>Swiercz</Author><Year>2008</Year><RecNum>14789</RecNum>><DisplayText>[75]</DisplayText><record><rec-number>14789</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evealxfs0err5sr" timestamp="1596039305">14789</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Swiercz, R.</author><author>Halatek, T.</author><author>Wasowicz, W.</author><author>Kur, B.</author><author>Grzelińska, Z.</author><author>Majcherek, W.</author></authors></contributors><auth-

address>Department of Toxicology and Carcinogenesis, Nofer Institute of Occupational Medicine, Łódź, Poland. radek@imp.lodz.pl</auth-address><titles><title>Pulmonary irritation after inhalation exposure to benzalkonium chloride in rats</title><secondary-title>Int J Occup Med Environ Health</secondary-title><alt-title>International journal of occupational medicine and environmental health</alt-title></titles><periodical><full-title>International journal of occupational medicine and environmental health</full-title><abbr-1>Int J Occup Med Environ Health</abbr-1></periodical><alt-periodical><full-title>International journal of occupational medicine and environmental health</full-title><abbr-1>Int J Occup Med Environ Health</abbr-1></alt-periodical><pages>157-63</pages><volume>21</volume><number>2</number><edition>2008/08/22</edition><keywords><keyword>Animals</keyword><keyword>Benzalkonium Compounds/administration & dosage/*toxicity</keyword><keyword>Female</keyword><keyword>Inhalation Exposure</keyword><keyword>Lung Diseases/*chemically induced/pathology</keyword><keyword>Organ Size/drug effects</keyword><keyword>Rats</keyword><keyword>Rats, Wistar</keyword></keywords><dates><year>2008</year></dates><isbn>1232-1087 (Print)1232-1087</isbn><accession-num>18715840</accession-num><urls></urls><electronic-resource-num>10.2478/v10001-008-0020-1</electronic-resource-num><remote-database-provider>NLM</remote-database-provider><language>eng</language></record></Cite></EndNote>]. The LC₅₀ was reported to be approximately 53 mg/m³ and BALF analysis reported increased inflammatory markers such as tumor necrosis factor (TNF)-α, interleukin (IL)-6. Indicators of respiratory tract damage, including increased LDH, total protein, and lung weight were also observed.

Three repeated dose inhalation studies of three different exposure durations were identified for DDAC: 14-day, 28-day, and 90-day.

In the 14-day study, male Sprague-Dawley rats were exposed *via* whole-body inhalation exposures to DDAC aerosols of 0.15 mg/m³, 0.6 mg/m³, and 3.6 mg/m³ for 6 hours/day, 7 days/week [ADDIN EN.CITE

<EndNote><Cite><Author>Lim</Author><Year>2014</Year><RecNum>14790</RecNum><DisplayText>[76]</DisplayText><record><rec-number>14790</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evealrxfds0err5sr" timestamp="1596039544">14790</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Lim, C. H.</author><author>Chung, Y. H.</author></authors></contributors><auth-address>Toxicity Research Team, Occupational Safety and Health Research Institute, KOSHA, Daejeon, Korea.</auth-address><titles><title>Effects of didecyldimethylammonium chloride on sprague-dawley rats after two weeks of inhalation exposure</title><secondary-title>Toxicol Res</secondary-title><alt-title>Toxicological research</alt-title></titles><periodical><full-title>Toxicol Res</full-title><abbr-1>Toxicological research</abbr-1></periodical><alt-periodical><full-title>Toxicol Res</full-title><abbr-1>Toxicological research</abbr-1></alt-periodical><pages>205-10</pages><volume>30</volume><number>3</number><edition>2014/10/25</edition><keywords><keyword>Biocide</keyword><keyword>Didecyldimethylammonium chloride</keyword><keyword>Inhalation</keyword></keywords><dates><year>2014</year><

pub-dates><date>Sep</date></pub-dates></dates><isbn>1976-8257 (Print)1976-8257</isbn><accession-num>25343015</accession-num><urls></urls><custom2>PMC4206748</custom2><electronic-resource-num>10.5487/tr.2014.30.3.205</electronic-resource-num><remote-database-provider>NLM</remote-database-provider><language>eng</language></record></Cite></EndNote>]. The study authors reported an MMAD of 1.86 μm and a GSD of 2.75; however, individual values for each exposure concentration were not provided. Mild effects were noted in cell differential counts and cell damage parameters in BALF, in addition to inflammatory cell infiltration, and interstitial pneumonia at the medium and high exposures. The NOAEC was determined to be 0.15 mg/m^3 .

In the intermediate exposure (4-week) study, male and female Sprague-Dawley rats (5 rats/sex/group) were exposed *via* dynamic nose-only inhalation to concentrations of 0, 0.08, 0.5, and 1.5 mg/m^3 DDAC (MMAD 1.4, 1.5, and 1.9 μm , GSD 1.83, 1.86, and 1.87, density not reported) for 6 hours/day, 5 days/week [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2016</Year><RecNum>14732</RecNum><DisplayText>[10]</DisplayText><record><rec-number>14732</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evealxfds0err5sr" timestamp="1596018482">14732</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Subchronic Inhalation Toxicity Study of DDAC - Revised</title><secondary-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington,

D.C. 20460</secondary-title></titles><periodical><full-title>Office of Chemical Safety and
Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-
title></periodical><pages>25</pages><volume>HQ-OPP-2006-0338-
0045</volume><dates><year>2016</year></dates><urls></urls></record></Cite></EndNote>]

. Body weights were significantly reduced in the high exposure group (males only) on days 14,
21, and 25. Lung weights were increased in females in the mid- and high-concentration groups
and in males in the high concentration group. BALF analysis indicated that, at the high
concentration, neutrophils and eosinophils increased with a concomitant decrease in
macrophages. Histopathological findings in the nasal cavity were graded according to severity
from minimal to severe and increased mucus of the respiratory epithelium in males and females
was minimal to moderate at all exposures and mild to moderate ulceration of the nasal cavity in
males and females in the high concentration group only. In males, there was an increase in cell
count and total protein across all exposures. In females, there was an increase in LDH across all
concentrations, but the small sample size precluded establishing statistical significance for the
effects. A conservative LOAEC of 0.08 mg/m³ was previously identified by the Agency based on
increased mucus of the respiratory epithelium and increased LDH; however, due to the mild
effects and low number of animals/group, the effects were not statistically significant [ADDIN
EN.CITE

<EndNote><Cite><Author>EPA</Author><Year>2016</Year><RecNum>14732</RecNum><
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In the 13-week sub-chronic study, male and female Sprague-Dawley rats (10/group/sex) were exposed in whole-body exposure chambers for 6 hours/day, 5 days/week [ADDIN EN.CITE <EndNote><Cite><Author>Kim</Author><Year>2017</Year><RecNum>14736</RecNum><DisplayText>[77]</DisplayText><record><rec-number>14736</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evealxfds0err5sr" timestamp="1596018905">14736</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Kim, Y. S.</author><author>Lee, S. B.</author><author>Lim, C. H.</author></authors></contributors><auth-address>Chronic Inhalation Toxicity Research Center, Chemicals Toxicity Research Bureau, Occupational Safety and Health Research Institute, KOSHA, Daejeon, Korea.</auth-address><titles><title>Effects of Didecyldimethylammonium Chloride (DDAC) on Sprague-Dawley Rats after 13 Weeks of Inhalation Exposure</title><secondary-title>Toxicol Res</secondary-title><alt-title>Toxicological research</alt-title></titles><periodical><full-title>Toxicol Res</full-title><abbr-1>Toxicological research</abbr-1></periodical><alt-periodical><full-title>Toxicol

Res</full-title><abbr-1>Toxicological research</abbr-1></alt-periodical><pages>7-14</pages><volume>33</volume><number>1</number><edition>2017/01/31</edition><keywords><keyword>Biocide</keyword><keyword>Didecyldimethylammonium chloride</keyword><keyword>Inhalation</keyword><keyword>Sub-chronic</keyword></keywords><dates><year>2017</year><pub-dates><date>Jan</date></pub-dates></dates><isbn>1976-8257 (Print)1976-8257</isbn><accession-num>28133508</accession-num><urls></urls><custom2>PMC5266374</custom2><electronic-resource-num>10.5487/tr.2017.33.1.007</electronic-resource-num><remote-database-provider>NLM</remote-database-provider><language>eng</language></record></Cite></EndNote>]. The MMAD of the DDAC aerosol was 0.63 μm , 0.81 μm , and 1.65 μm , and the geometric standard deviations were 1.62, 1.65, and 1.65 in the low ($0.11 \pm 0.06 \text{ mg/m}^3$), the middle ($0.36 \pm 0.20 \text{ mg/m}^3$) and the high ($1.41 \pm 0.71 \text{ mg/m}^3$) exposure groups, respectively. Body weight influenced by exposure to DDAC with the mean body weight approximately 35% lower in the high exposure ($1.41 \pm 0.71 \text{ mg/m}^3$) male group and 15% lower in the high exposure ($1.41 \pm 0.71 \text{ mg/m}^3$) female group compared to that of the control group. Albumin and LDH were unaffected in the BALF. Lung weight was increased in females in the mid- and high-concentration groups and in males in the high concentration group only, while inflammatory cell infiltration and interstitial pneumonia was observed in both the mid- and high-concentration groups. Tidal volume and minute volume were not significantly affected at any concentration. Severe histopathological symptoms such as proteinosis and/or fibrosis, were not reported. A NOAEC of 0.11 mg/m^3 was identified based on the increased lung weights in females and increase in inflammatory cells.

BAC was evaluated in a 2-week whole-body inhalation study in male and female Fischer rats (5/group/sex) to concentrations of 0.8, 4 and 20 mg/m³ for 6 hours/day, 7 days/week [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Mean concentration of BAC in the whole-body exposure chambers of the T1 (0.8 mg/m³), T2 (4 mg/m³) and T3 (20 mg/m³) groups during the exposure period was 0.84 ± 0.09, 4.01 ± 0.12, and 19.57 ± 0.97 mg/m³, respectively; the MMAD of the aerosols was 1.614, 1.090, and 1.215 µm, respectively, and the GSD was 2.00, 1.86, and 1.51, respectively. The MMAD and GSD were confirmed to be within the range recommended by the OECD (2018) [ADDIN EN.CITE

<EndNote><Cite><Author>OECD</Author><Year>2018</Year><RecNum>14819</RecNum><DisplayText>[79]</DisplayText><record><rec-number>14819</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evealxfs0err5sr" timestamp="1596046851">14819</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Guidance Document on Inhalation Toxicity Studies, Series on Testing and Assessment, No. 39 (Second Edition)</title><secondary-title>Environment Directorate, Joint Meeting of the Chemicals Committee and The Working Party on Chemicals, Pesticides and Biotechnology, Organization for Economic Cooperation and Development</secondary-title></titles><periodical><full-title>Environment Directorate, Joint Meeting of the Chemicals Committee and The Working Party on Chemicals, Pesticides and Biotechnology, Organization for Economic Cooperation and Development</full-title></periodical><pages>106, [https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono\(2009\)28/rev1&doclanguage=en](https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2009)28/rev1&doclanguage=en)</pages><volume>ENV/JM/MONO(2009)28/REV1</volume><dates><year>2018</year></dates><urls></urls></record></Cite></EndNote>]. Among the general signs observed

during the exposure period, soiled perineal region, rales, and discharge were continuously observed during the 2-week recovery period. Rales and deep respiration were observed in the high concentration. Exposure-related effects in the upper airway included nasal discharge at the low and mid concentrations, and ulceration with suppurative inflammation, squamous metaplasia, and erosion with necrosis were observed in the respiratory epithelium and transitional epithelium of the male and female high concentrations.

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In the lower airways, degeneration and regeneration of terminal bronchiolar epithelium, smooth muscle hypertrophy of bronchioloalveolar junction, and cell debris in the alveolar lumens were observed in the mid and high concentration male groups and high concentration dose female group. Hypertrophy and hyperplasia of mucous cells in the bronchi or bronchioles were observed in both males and females. Effects indicating tissue injury included squamous metaplasia of the respiratory epithelium and transitional epithelium, mucinous cell hypertrophy and proliferation of the respiratory epithelium, mucinous cell metaplasia of the transitional epithelium in the nasal cavities, and mucinous cell hypertrophy and proliferation of terminal bronchiole. In the BALF analysis, the concentration of reactive oxygen species (ROS)/reactive nitrogen species (RNS), IL-1 β , IL-6, and macrophage inflammatory protein (MIP)-2 decreased concentration-dependently at the end of the exposure period, which indicated oxidative damage, but did not show a concentration-dependent change at 4 weeks of recovery. The concentrations of TNF- α , IL-4, and transforming growth factor (TGF)- β did not show changes associated with test substance exposure. Relative lung weights were statistically significantly increased in males at the mid and high doses and in females at the high doses only. The study authors identified a LOAEC of 0.8 mg/m³ based on effects in the nasal cavity.

Mechanistic studies

In vitro assays have demonstrated that cytotoxic effects of cationic surfactants have significantly greater toxicity to non-polarized than polarized mammalian cells [ADDIN EN.CITE ADDIN EN.CITE.DATA]. In this study, cell viability as measured by LDH and MTT assays in non-polarized HeLa immortal cell line cells and fetal skin dendritic cells (FSDC) was more sensitive to the effects of different cationic surfactants of varying alkyl chain length and polar head groups than polarized cell lines Madin-Darby Canine Kidney (MDCK) and Caco-2. The cationic surfactant toxicity was shown to occur well below their CMC, and greater toxicity was observed with alkyl lengths of 10-12 than 14-16; however, this association was not strictly a linear relationship. In addition, the cationic surfactants with a larger polar head group (*i.e.*, benzalkonium) were 2-5 times more toxic than cationic surfactants with a more localized charge (*i.e.*, trimethylammonium).

The effects of BAC on cell viability, inflammatory response, and oxidative stress of human alveolar epithelial cells has been replicated *in vitro* using a dynamic culture condition that reflects the natural microenvironment of the lung to simulate the contraction and expansion of breathing [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Normal breathing levels were simulated (tidal volume 10%, 0.2Hz) through surface elongation of an elastic membrane in a dynamic culture system. This type of dynamic system provided easy control of exposure rate during the cell culture. The system assessed toxicity by culturing submerged cells with different BAC concentrations (0, 2, 5, 10, 20, and 40 µg/mL) under static and dynamic culture conditions. Following a 24-hr exposure to BAC, cellular metabolic activity, IL-8, and ROS levels were

significantly affected, compared to untreated cells, when using either static or dynamic cell growth conditions. The dynamic culture system, which more closely mimics lung conditions, showed a higher toxic response to BAC as indicated by increased ROS levels.

Dose-Response Analysis: Quantitative Points of Departure (PODs)

The animal inhalation toxicity data identified by the literature search and PODs from the studies are summarized in [REF _Ref46931035 \h * MERGEFORMAT]. It should be emphasized that new information (*e.g.*, study data, POD derivation approaches, mechanistic information, *etc.*) may lead to updates/additions to this table. All of the identified data are from animal studies and therefore need to be extrapolated to estimate the human equivalent inhalation exposure [ADDIN EN.CITE

<EndNote><Cite><Author>EPA</Author><Year>1994</Year><RecNum>14746</RecNum><DisplayText>[20]</DisplayText><record><rec-number>14746</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evealxfs0err5sr" timestamp="1596021628">14746</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry</title><secondary-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</secondary-title></titles><periodical><full-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</full-title></periodical><pages>389, [\[PAGE \]](https://www.epa.gov/sites/production/files/2014-</p>
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11/documents/rfc_methodology.pdf</pages><volume>EPA/600/8-

90/066F</volume><dates><year>1994</year></dates><urls></urls></record></Cite></EndNot

e>]. The exposure duration adjustment and DAF approaches were described above. The

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summary of RDDR inputs (e.g., MMAD and GSD) and results are provided in [REF

_Ref46931035 \h * MERGEFORMAT] for each of the toxicity studies from which PODs

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could be identified. However, other approaches to dosimetry adjustment may be considered relevant (e.g., use of the multiple-path particle dosimetry model [MPPD]).

For the nonionic surfactant, octylphenoxypolyethoxyethanol, the effects observed (increased lung weights, alveolar/bronchiolar epithelial hyperplasia and lung inflammation) are consistent with effects in the thoracic region; therefore, the RDDR of 0.812 was used to calculate the HEC.

For the anionic surfactant, oleoylsarcosine, the effects were seen in multiple regions of the respiratory tract, including squamous metaplasia and epithelium proliferation and submucous acute inflammation at the base of the epiglottis and early stages of fibrosis in the alveoli walls.

Therefore, the extrathoracic RDDR (0.0111) was used to calculate the HEC. In the 28-day inhalation study with DDAC, effects were observed throughout the respiratory tract, including the nasal cavity; therefore, the thoracic RDDR (0.854) was used for calculating the HEC.

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Similarly, for the cationic surfactant, BAC histopathological cellular changes were observed in the nasal cavity and lungs, indicating the extrathoracic RDDR (0.106) should be used to calculate the HEC. The RDDRs applied and HECs derived from the animal study PODs are provided in [

REF _Ref46931035 \h * MERGEFORMAT].

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Table [SEQ Table * ARABIC]. Inhalation Toxicity Points of Departure and Human Equivalent Concentrations (HEC) for Surfactants.

Surfactant Type	Chemical Substance	Inhalation Exposure Duration/Type	Study POD	Value (mg/m ³)	Reference	Density (g/cm ³) at 20 °C ¹	RDDR Model Input Parameters		RDDR ²	HEC (mg/m ³)
							MM AD (μm)	GSD		
Nonionic	octylphenoxypolyethoxyethanol (CASRN 9002-93-1)	14-day, 6 hr/d, 5 d/wk; whole body	LOAEC	5.3	[ADDIN EN.CITE <EndNote><Cite><Author>MDEQ </Author><Year>2003</Year><RecordNum>14731</RecordNum><DisplayText>[8]</DisplayText><record><rec-number	0.998 water vehicle	1.80	1.80	RDDR _{ET} = 0.196 RDDR _{TB} = 1.367 RDDR _{PU} = 0.564 RDDR_{TH} = 0.812 RDDR _{TOT} = 1.547	1.0 7.2 3.0 4.4 8.2

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					<author>MD EQ</author></ authors ></contributors ><titles ><title >To: Memo to File for Triton X-100 (CAS # 9002- 93-1); From: Gary Butterfi eld; Date: Novem ber 21,200 3; Subject : Screeni ng level for Triton					
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					X-100 (CAS # 9002- 93- 1)</title ><seco ndary- title>M ichigan Depart ment of Environ mental Quality (MDE Q)</sec ondary- title></ titles>< periodi cal><fu ll- title>M ichigan Depart ment of Environ mental Quality (MDE Q)</ful l- title></					
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					periodical><pages>2</pages><dates><year>2003</year></dates><urls></urls></record></Cite></EndNote>]					
Anionic	oleoyl sarcosine (CASRN 110-25-8)	28-day, 6 hr/d, 5 d/wk; nose-only (OECD TG 412)	LOAEC	< 6	[ADDIN EN.CITE <EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecordNumber>14784</RecordNumber><Disclaimer></Disclaimer>	0.7893 ethanol vehicle	1.16	2.12	RDDR_{ET} = 0.111 RDDR _{TB} = 2.008 RDDR _{PU} = 0.447 RDDR _{TH} = 0.742 RDDR _{TOT} = 0.970	< 0.6 < 12.0 < 2.7 < 4.5 < 5.8

					splayText>[62] </DisplayText><record><record-number>14784</record-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596036869">14784</key></foreign-keys><ref-type name="Journal					
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Cationi c	DDAC	4-week, 6 hr/d, 5 d/wk; nose-only	LOAE C ³ (lung effects)	0.08	[ADDIN EN.CIT E <EndN ote><C ite><A uthor> EPA</ Author ><Year >2016< /Year>	NR	1.60	1.85	RDDR _{ET} = 0.211 RDDR _{TB} = 1.674 RDDR _{PU} = 0.539 RDDR_{TH} = 0.854 RDDR _{TOT} = 1.607	0.02 0.13 0.04 0.07 0.13

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	BAC	14-day, 6 hr/d, 7 d/wk; whole body	LOAE C (nasal effects)	0.8	[ADDIN EN.CIT E ADDIN EN.CIT E.DAT A]	0.998 water vehicle 2% dose solution	1.31	1.79	RDDR_{ET} = 0.106 RDDR _{TB} = 1.988 RDDR _{PU} = 0.528 RDDR _{TH} = 0.815 RDDR _{TOT} = 0.991	0.08 1.59 0.42 0.65 0.79

MMAD: Mass Median Aerodynamic Diameter of inhalation study aerosol, average values listed; GSD: Geometric Standard Deviation of the inhalation study aerosol, average values listed; RDDR: Regional Deposited Dose Ratio; ET: Extrathoracic; TB: Tracheobronchial; PU: Pulmonary; TH: Thoracic = TB + PU; TOT = ET + TB + PU.

¹Exact density of administered compounds not reported (NR); vehicle density was listed when provided.

²RDDR values are for male and female animals, whichever was lower, as calculated using RDDR.exe and described in the Supporting Information file at “Section 2 RDDR Modeling”.

³conservative estimate: effects were not statistically significant.

NA: Data not available or RDDR values could not be calculated from the available information.

Benchmark Margin of Exposure Analysis

The substances shown in [REF _Ref46931035 \h * MERGEFORMAT] provide representative examples of PODs that may be applied to new chemistries that meet the Surfactant Criteria, after evaluating whether the chemical substances in [REF _Ref46931035 \h * MERGEFORMAT] are appropriate toxicological analogues for read-across to the new chemical substance.

Alternatively, the notifier may propose a different representative POD and/or analogue, if supported by scientific evidence. If a determination cannot be made on whether one of these chemical substances ([REF _Ref46931035 \h * MERGEFORMAT] or other representative analogue) is an appropriate toxicological analogue, then the relevant substance from [REF _Ref46931035 \h * MERGEFORMAT] should be identified as a comparator substance⁴ for use in the Tiered-Testing Strategy, discussed below. Though the initial starting point for deriving a benchmark MOE is based on a composite of the default values of 10 for each of the individual values for UF_H, UF_A, and UF_L, refinements may be warranted based on dosimetric adjustments to the applied concentrations used for establishing the experimental PODs or consideration of the representativeness and comprehensiveness of the available database to characterize potential effects after inhalation exposure. As shown in [REF _Ref46931035 \h * MERGEFORMAT], the uncertainty factors were based on RDDRs that were used as DAFs to account for animal-to-human toxicokinetic differences.

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⁴ A comparator substance is one that may possess similar properties to the new chemical substance and for which inhalation toxicity data are available. EPA may “read-across” the toxicity data from the comparator substance to the new chemical substance when no other information is available. The tiered-testing approach for this category is designed to determine whether this practice may be refined or supported by additional data. As such, the comparator substance should be used in side-by-side testing in Tiers I-III with a new chemical substance to aid with interpreting the test results of the new chemical substance.

In the case of surface-active substances meeting the Surfactant Criteria, EPA has recently adopted a generalized approach that has historically been applied on a case-by-case basis for chemical substances, in recognition that surface-active effects that lead to irritation/corrosion do not require absorption, metabolism, distribution, or elimination (ADME) (See, *e.g.*, EPA, 2020 [ADDIN EN.CITE

<EndNote><Cite><Author>EPA</Author><Year>2020</Year><RecNum>14794</RecNum><DisplayText>[82]</DisplayText><record><rec-number>14794</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfs0err5sr" timestamp="1596040494">14794</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Hazard Characterization of Isothiazolinones in Support of FIFRA Registration Review</title><secondary-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>84, https://www.regulations.gov/contentStreamer?documentId=EPA-HQ-OPP-2013-0605-0051&contenttype=pdf</pages><volume>EPA-HQ-OPP-2013-0605-0051</volume><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]

). In the context of this publication, irritation/corrosion include those effects in the respiratory tract that lead to inflammation, hyperplasia, and metaplasia. For chemical substances that act *via* a direct-acting adverse outcome pathway (AOP) mode of action (MOA) such as the one

Commented [A31]: Changing from AOP (which is for sure used as specific wording up to day only in limited cases – e.g. sensitisation) to MoA would break the connection to AOP described in the later section.

I we want to change this we should explain how the connection from AOP to MoA is.

For me AOP is always a complete path e.g the animal cant breath due to key events irritation cell death etc. So the AOP gives the whole picture with the adverse effect at the end whereas MoA could be for me a local irritation... this may than lead to breathing difficulties as well but this is not covered within this, it is for me secondary to the local irritation.

Therefore I would appreciate if we could explain the connection to AOP as we use this later in our manuscript if we need to change the wording here.
Will be happy to discuss this.

regarding surfactant that is under development [ADDIN EN.CITE

<EndNote><Cite><Author>Sorli</Author><Year>2020</Year><RecNum>14800</RecNum><

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Article">17</ref-type><contributors><authors><author>Sorli, J.

B.</author></authors></contributors><titles><title>Lung Surfactant Function Disruption

Leading to Acute Inhalation Toxicity</title><secondary-title>AOPWiki</secondary-

title></titles><periodical><full-title>AOPWiki</full-

title></periodical><pages>https://aopwiki.org/aops/302</pages><dates><year>2020</year></d

ates><urls></urls></record></Cite></EndNote>], the default values for UF_H and UF_A are each

reduced to 3 (*i.e.*, $10^{0.5}$ or 3.162) to account for the uncertainty/variability for toxicodynamics,

whereas the toxicokinetic component is reduced to 1. In order to apply these reductions, the

following criteria must be established:

1. A description of the MOAAOP,
2. A discussion of why the MOAAOP is unlikely to differ between humans, in the case of UF_H , or between animals in comparison to humans, in the case of UF_A , and
3. A discussion as to why the ADME of the chemical substance is addressed by the use of dosimetry modeling.

When the above criteria are met, application of the appropriate DAF (*e.g.*, the RDDR for particles) should still be applied, given that deposition is the most appropriate dose metric for

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assessing acute/subacute effects from surface-active agents. However, since the DAF accounts for the toxicokinetic component of UF_A , the remaining value of 3 (*i.e.*, $10^{0.5}$ or 3.16) should be retained for the toxicodynamics component of the UF_A .

Based on these information and criteria, the following composite values are appropriate to describe intra- and interspecies variability (*i.e.*, $UF_H \times UF_A$):

$UF_H = 10$ or 3: The default value of 10 should be applied when the available information does not support each of the above criteria. If the available information supports all three of the above criteria, then a value of 3 may be applied, which reflects ~~The reduced value represents a~~ reduction in the TK component of this UF to 1 and application of a value of 3 for the ~~with the~~ remaining value of 3 accounting for the TD component.

$UF_A = 10$ or 3: The default value of 10 should be applied when the available information does not support the application of dosimetric adjustments for ~~quantifying deriving an~~ HEC or when ~~the available information~~ does not support each of the above three criteria. If the available information allows derivation of an HEC and/or application of the above criteria, then a value of 3 may be applied, which ~~reflects~~ presents a reduction in the TK component to 1 and application of a value of 3 for the TD component.

$UF_L = 10$ or 1: If the POD from the experimental study is based on a LOAEC, then a default value of 10 should be applied, unless there is information to support that a reduced value is warranted. If the experimental data are amenable to benchmark dose modeling, a BMCL with an

appropriate biologically significant benchmark response (e.g., 10% extra risk for quantal data or 1 standard deviation for continuous data) should be calculated and a value of 1 should be assigned to this applied for this area of uncertainty factor.

The above considerations and approaches support the application of a benchmark MOE ranging from 10 (i.e., $10^{0.5} \times 10^{0.5} \approx 10$) to 1,000 depending on the chemical substance identified as an appropriate toxicological analogue and available data on the new chemical substance. In those instances where the data are too limited to determine whether one of the chemical substances in Table 3 is appropriate for extrapolating the hazards to the new chemical substance, experimental testing should be performed to aid with informing the quantitative assessment, as discussed under the Tiered-Testing Strategy.

Uncertainties and Limitations

Commented [A32]: Seems very long

The assessment framework outlined includes a number of uncertainties and limitations, including those associated with extrapolating the hazards identified from the chemical substances shown in [REF _Ref46931035 \h * MERGEFORMAT]. Uncertainties associated with using animals to estimate human toxicity are recognized and methods are presented to reduce extrapolation uncertainties [ADDIN EN.CITE

<EndNote><Cite><Author>OECD</Author><Year>2014</Year><RecNum>14795</RecNum>
<DisplayText>[84]</DisplayText><record><rec-number>14795</rec-number><foreign-
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type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Guidance on Grouping of Chemicals, Second Edition, Series on Testing & Assessment</title><secondary-title>Environment Directorate, Joint Meeting of the Chemicals Committee and The Working Party on Chemicals, Pesticides and Biotechnology, Organization for Economic Cooperation and Development</secondary-title></titles><periodical><full-title>Environment Directorate, Joint Meeting of the Chemicals Committee and The Working Party on Chemicals, Pesticides and Biotechnology, Organization for Economic Cooperation and Development</full-title></periodical><pages>141, [http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono\(2014\)4&doclanguage=en](http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2014)4&doclanguage=en)</pages><volume>ENV/JN/MONO(2014)4</volume><dates><year>2014</year></dates><urls></urls></record></Cite></EndNote>]. Procedures for the adjustment of exposure durations for inhalation exposures and application of DAFs to derive HECs are well-established procedures for reducing uncertainties associated with the TK aspects of animal-to-human extrapolation factors and derivation of benchmark MOEs (i.e., type and magnitude of uncertainty factors) [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2002</Year><RecNum>14743</RecNum><DisplayText>[19, 20]</DisplayText><record><rec-number>14743</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evealxfds0err5sr" timestamp="1596019884">14743</key></foreign-keys><ref-type name="Journal Article">17</ref-type></contributors><authors><author>EPA</author></authors></contributors><titles><title>A Review of the Reference Dose and Reference Concentration Processes</title><secondary-title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C.

20460</secondary-title></titles><periodical><full-title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>192, <https://www.epa.gov/sites/production/files/2014-12/documents/rfd-final.pdf></pages><volume>EPA/630/P-02/002F</volume><dates><year>2002</year></dates><urls></urls></record></Cite><Cite><Author>EPA</Author><Year>1994</Year><RecNum>14746</RecNum><record><rec-number>14746</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596021628">14746</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry</title><secondary-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</secondary-title></titles><periodical><full-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</full-title></periodical><pages>389, https://www.epa.gov/sites/production/files/2014-11/documents/rfc_methodology.pdf</pages><volume>EPA/600/8-90/066F</volume><dates><year>1994</year></dates><urls></urls></record></Cite></EndNote>]. Likewise, EPA ~~has recommends~~ed that BMD modeling be employed whenever possible to identify a POD and to reduce uncertainties associated with using a LOAEL from a toxicity study.

Given the small number of chemical substances that meet the Surfactant Criteria that have concentration-response inhalation toxicity data, the applicability of the chemical substances in [

REF _Ref46931035 \h * MERGEFORMAT] to new chemical substances needs to be carefully considered, with attention given to the influence of additional functional groups on the toxicity of the new chemical substance, as well as the particle properties (MMAD, GSD, and density) of the candidate new chemical substance. Simulation studies using dosimetry models such as the RDDR or multiple-path particle dosimetry (MPPD) models can inform these considerations. Additionally, the risk assessors should consider if a different comparator substance and/or POD may be more appropriate (*e.g.*, based on new scientific information of the new chemical substance profile). Risk assessors should consider the surface tension and CMC criteria ([REF _Ref47613375 \h * MERGEFORMAT]) compared to these measurements for the new chemical substance and the influence of the presence or absence of additional functional groups on these criteria (*e.g.*, would a particular functional group increase or decrease toxicity, for example by another mechanism of action). If such structural differences are judged not to significantly influence properties and toxicity, such that the new chemical substance is expected to have comparable or lower toxicity, the hazard(s) and risk(s) should be characterized using the chemical substance as a toxicological analogue to the new chemical substance. Of course, uncertainties regarding this extrapolation should be acknowledged in the risk characterization.

For instances where the notifier of the new chemical substance and/or EPA is unable to conclude that a chemical substances ([REF _Ref46931035 \h * MERGEFORMAT]) or other relevant analogue) is comparable to or represents an acceptable toxicological analogue to the new chemical substance, then the Tiered-Testing Strategy provided could be used to determine whether the new chemical substance has lower, comparable, or higher toxicity to the relevant chemical substance in [REF _Ref46931035 \h * MERGEFORMAT], as a comparator

substance and not as a toxicological analogue. Prior to conducting such testing, the scientific basis for selecting the comparator substance to the new chemical substance should be understood and a rationale provided as to why the comparator substance will be used for testing.

Use of New Approach Methods (NAMs) and *In Vitro* Testing Strategies to Reduce or Replace Vertebrate Testing

The amended TSCA requires EPA to reduce reliance on animal testing using methods and strategies that “provide information of equivalent or better scientific quality and relevance for assessing risks of injury to health or the environment” [ADDIN EN.CITE

<EndNote><Cite><Author>U.S.C.</Author><Year>2016</Year><RecNum>14796</RecNum>

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Title 15-Commerce and Trade, Chapter 53-Toxic Substances Control, Subchapter I-Control of

Toxic Substances</title><secondary-title>United States Code (U.S.C.)</secondary-

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title></periodical><pages>https://uscode.house.gov/view.xhtml?path=/prelim@title15/chapter53

&edition=prelim</pages><dates><year>2016</year></dates><urls></urls></record></Cit

e></EndNote>]. Moreover, the amended TSCA requires entities undertaking voluntary testing

for submission to EPA to first “...attempt to develop the information by means of an alternative

test method or strategy ...before conducting new vertebrate testing...” [ADDIN EN.CITE

Commented [A33]: Shorten?:

Amy, William, Jane

Reviewer #1 wanted even more explanation of test methods, so not shortening this section much

<EndNote><Cite><Author>U.S.C./<Author><Year>2016/<Year><RecNum>14796/<RecNum>
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 title></periodical><pages>https://uscode.house.gov/view.xhtml?path=/prelim@title15/chapter53
 &edition=prelim/<pages><dates><year>2016/<year></dates><urls></urls></record></Cit
 e></EndNote>]. Additionally, in 2019, EPA was directed to prioritize efforts to use NAMs to
 reduce animal testing [ADDIN EN.CITE
 <EndNote><Cite><Author>Wheeler/<Author><Year>2019/<Year><RecNum>14797/<RecNu
 m><DisplayText>[86]/<DisplayText><record><rec-number>14797/<rec-number><foreign-
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 A.R./<author></authors></contributors><titles><title>Directive to Prioritize Effects to Reduce
 Animal Testing/<title><secondary-title>United States Environmental Protection
 Agency/<secondary-title></titles><periodical><full-title>United States Environmental
 Protection Agency/<full-title></periodical><pages>3,
 https://www.epa.gov/sites/production/files/2019-09/documents/image2019-09-

231249.pdf</pages><dates><year>2019</year></dates><urls></urls></record></Cite></EndN

ote>]. Multiple NAMs exist which can be used to assess hazards and risks of new chemical substances that meet the Surfactant Criteria, including validated OECD methods for *in vitro* irritation testing and *in vitro* methods to specifically assess respiratory toxicity. Several methods are described within a tiered-testing strategy recognizing that these assays are provided as examples and the development of NAMs is advancing rapidly. As such, the NAMs included here should not be considered all-inclusive or a final compilation. EPA strongly encourages the development and use of NAMs, particularly to reduce or replace the use of animals and is open to considering and discussing additional NAMs with PMN submitters during a pre-notice consultation [ADDIN EN.CITE

<EndNote><Cite><Author>EPA</Author><Year>2020</Year><RecNum>14829</RecNum><

DisplayText>[87]</DisplayText><record><rec-number>14829</rec-number><foreign-

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chedule a Pre-Submission Meeting, Reviewing New Chemicals under the Toxic Substances

Control Act (TSCA)</title><secondary-title>Office of Pollution Prevention and Toxics, U.S.

Environmental Protection Agency, Washington, D.C. 20460</secondary-

title></titles><periodical><full-title>Office of Pollution Prevention and Toxics, U.S.

Environmental Protection Agency, Washington, D.C. 20460</full-

title></periodical><pages>https://www.epa.gov/reviewing-new-chemicals-under-toxic-

substances-control-act-tsca/forms/program-contacts-

and</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>].

In the interest of reducing or replacing vertebrate testing and designing a scientifically robust testing approach, when a surfactant is determined to be respirable, EPA encourages evaluating its potential to cause respiratory tract toxicity using an AOP approach. The OECD provides “An AOP is an analytical construct that describes a sequential chain of causally linked events at different levels of biological organization that lead to an adverse health or ecotoxicological effect” and that “AOPs are the central element of a toxicological knowledge framework being built to support chemical risk assessment based on mechanistic reasoning” [ADDIN EN.CITE

<EndNote><Cite><Author>OECD</Author><Year>2020</Year><RecNum>14798</RecNum>

<DisplayText>[88]</DisplayText><record><rec-number>14798</rec-number><foreign-

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(OECD)</full-title></periodical><pages>http://www.oecd.org/env/ehs/testing/adverse-outcome-

pathways-molecular-screening-and-

toxicogenomics.htm</pages><dates><year>2020</year></dates><urls></urls></record></Cite

></EndNote>]. AOPs in various stages of development are useful for different purposes and an AOP may be useful even if it has not been formally evaluated by the OECD.

An AOP can be used to help design a testing strategy and to identify NAMs that can query the key events leading up to the adverse outcome. As an example, using the respiratory contact irritant chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile; CASRN 1897-45-6), Syngenta Crop Protection applied a NAM for the assessment of inhalation toxicology based on an AOP approach [ADDIN EN.CITE ADDIN EN.CITE.DATA]. The approach involved derivation of the POD from an *in vitro* assay and the integration of the *in vitro* POD for calculation of HECs for the inhalation risk assessment. Similar approaches can be used for surfactants where *in vitro/ex vivo* systems may be used to investigate specific key events in an AOP and confirm that a new chemical substance fits within the boundaries of the Surfactant Category, and therefore, may act like a surfactant (group assignment *via* similar AOP) and/or if other substance-specific properties lead to a predominant type of key event within the AOP. Further, *in vitro* tests may deliver information while avoiding *in vivo* testing or, if considered, provide helpful information on dose-selection for *in vivo* testing.

An AOP connects a molecular initiating event (MIE) to key events, at the cellular, tissue, and organ levels, which lead to an adverse outcome at the organism or population level [ADDIN EN.CITE ADDIN EN.CITE.DATA]. For surfactants, proposed MIEs include interaction of the substance with the epithelial lining fluid or lung-surfactant, or the molecular interaction of the substance itself with cell membranes of the epithelium in the respiratory tract. The resulting key events include disruption of airway epithelial cells (AEC) due to loss of lung cell surfactant

function and/or the loss of membrane integrity (cellular level key events). These cellular events may lead to different tissue or organ level events (*e.g.*, cytotoxicity and perturbation of AEC, increased alveolar surface tension and alveolar collapse, loss of barrier function, blood extravasation, and impaired oxygenation of blood), which may finally lead to organism consequences (*i.e.*, the adverse outcome) (*e.g.*, pneumonia, limited lung function by chronic obstruction (COPD), interstitial fibrosis, *etc.*).

Commented [A34]: Define CLE here?

Some *in vitro* tests, such as by capillary surfactometer, may be useful in screening chemicals to be tested for the Surfactant Category, but do not by themselves constitute adequate tests for acute respiratory tract effects of these chemicals. This information should be taken into consideration within an integrated approach. These assays can be used as part of a weight of evidence evaluation to determine whether to consider animal testing or if a POD can be determined for risk assessment purposes without the use of animals. Each test can provide insight on one key event of the AOP, which collectively, may provide a comprehensive picture of the likelihood of toxicity.

A number of different types of *in vitro* test methods, summarized in [REF_Ref46931271 \h * MERGEFORMAT], may be used to query key events in AOPs relevant to the disruption of lung function by surfactants [ADDIN EN.CITE

<EndNote><Cite><Author>Sorli</Author><Year>2020</Year><RecNum>14800</RecNum><DisplayText>[83]</DisplayText><record><rec-number>14800</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evealrxfds0err5sr" timestamp="1596041625">14800</key></foreign-keys><ref-type name="Journal